

LEGIONELLA

and the prevention of legionellosis

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LEGIONELLA

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Edited by:

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Foreword

Legionellosis is a collection of infections that emerged in the second half of the 20th century, and that are caused by *Legionella pneumophila* and related *Legionella* bacteria. The severity of legionellosis varies from mild febrile illness (Pontiac fever) to a potentially fatal form of pneumonia (Legionnaires' disease) that can affect anyone, but principally affects those who are susceptible due to age, illness, immunosuppression or other risk factors, such as smoking. Water is the major natural reservoir for legionellae, and the bacteria are found worldwide in many different natural and artificial aquatic environments, such as cooling towers; water systems in hotels, homes, ships and factories; respiratory therapy equipment; fountains; misting devices; and spa pools. About 20% of the cases of legionellosis detected in Europe are considered to be travel-related; these cases present a particular set of problems because of difficulties in identifying the source of infection.

The World Health Organization (WHO) currently provides guidance on *Legionella* risk assessment and management in three principal documents:

- *Guidelines for Drinking-water Quality* (WHO, 2004)
- *Guidelines for Safe Recreational Water Environments* (WHO, 2006)
- *Guide to Ship Sanitation* (WHO, 2007).

As part of the ongoing review of the *Guidelines for Drinking-water Quality*, specific micro-organisms and chemicals are periodically evaluated, and documentation relating to protection and control of drinking-water quality is prepared. In 2001, a meeting was held in Adelaide, Australia, to discuss approaches to regulating microbial drinking-water quality, and development of risk assessment and risk management approaches, for incorporation into the 3rd edition of the *Guidelines for Drinking-water Quality* (WHO, 2004). At that meeting, health concerns relating to *Legionella* were identified as an area of increasing public and professional interest. The meeting recommended the development of this publication — *Legionella and the Prevention of Legionellosis* — to review the current state of knowledge about the impact of *Legionella* on health.

This book provides a comprehensive overview of the sources, ecology and laboratory identification of *Legionella*. It provides guidance on assessment and management of risks associated with potentially hazardous environments, such as cooling towers, pools and spa baths. The document also identifies necessary measures to prevent, or adequately control, the risk of exposure to *Legionella* bacteria for each particular environment. Outbreaks of legionellosis generally cause a high level of morbidity and mortality in the people exposed; therefore, the suspicion of an outbreak warrants immediate action. This publication reviews policies and practice for outbreak management and the institutional roles and responsibilities of an outbreak control team.

The development of this publication was guided by the recommendations of an expert meeting hosted by the Health Protection Agency's Centre for Infections (formerly the Central Public Health Laboratory), Colindale, London, on 18–20 June 2002, chaired by Dr John V Lee. It was also guided by a series of critical reviews undertaken by specialists in the field.

The production of this document was led by the Department of Public Health and Environment — Programme on Assessing and Managing Environmental Risks to Health at WHO, in cooperation with the Department of Epidemic and Pandemic Alert and Response at WHO.

This book will be useful to all those concerned with *Legionella* and health, including environmental and public health officers, health-care workers, the travel industry, researchers and special interest groups.

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Abbreviations and acronyms

AFLP	amplified fragment length polymorphism
AIDS	acquired immunodeficiency syndrome
BAL	bronchoalveolar lavage
BCYE	buffered charcoal yeast extract
CAP	community-acquired pneumonia
CFU	colony-forming unit
DFA	direct immunofluorescence assay
DNA	deoxyribonucleic acid
EIA	enzyme immunoassay
EWGLI	European Working Group for <i>Legionella</i> Infections
GP	general practitioner
HEPA	high efficiency particulate absorbing
HPC	heterotrophic plate count
IFAT	immunofluorescent antibody test
ISO	International Organization for Standardization
LLAP	<i>Legionella</i> -like amoebal pathogen
MAb	monoclonal antibody
Mip	macrophage infectivity potentiator
MOMP	major outer membrane protein
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PHLS	Public Health Laboratory Service (UK)
ppGpp	guanosine 3',5'-bispyrophosphate
RNA	ribonucleic acid
UV	ultraviolet
WHO	World Health Organization
WSP	water safety plan

Executive summary

Legionellosis is a collection of infections that emerged in the second half of the 20th century, and that are caused by *Legionella pneumophila* and related bacteria. The severity of legionellosis varies from mild febrile illness (Pontiac fever) to a potentially fatal form of pneumonia (Legionnaires' disease) that can affect anyone, but principally affects those who are susceptible due to age, illness, immunosuppression and other risk factors, such as smoking.

Legionella is an important pathogen in health-care acquired (nosocomial) pneumonia, particularly in immunocompromised patients. *Legionella* spp. can also cause community-acquired pneumonia, which has a high rate of hospital admission. Legionnaires' disease is recognized as a major form of travel-associated pneumonia, and about 20% of the cases of legionellosis detected in Europe are considered to be related to travel; these cases present a particular set of problems because of difficulties in identifying the source of infection. Although *Legionella* is a well-recognized problem in developed nations, data are scarce from developing countries. Since risk environments and susceptible populations are found worldwide, it is likely that the problem of *Legionella* is under-appreciated in developing countries.

Chapter 1 describes the disease types caused by *Legionella* bacteria, including risk factors, prevalence and outcomes of Legionnaires' disease. Although all *Legionella* species are considered potentially pathogenic for humans, *Legionella pneumophila* is the aetiological agent responsible for most reported cases of Legionnaires' disease.

Chapter 2 discusses the ecology and environmental sources of *Legionella*. Water is the major natural reservoir for legionellae, and the bacteria are found worldwide in many different natural and artificial aquatic environments and ranges of environmental conditions, such as cooling towers; water systems in hotels, homes, ships and factories; respiratory therapy equipment; fountains; misting devices; and spa pools.

The fact that legionellae are found in hot-water tanks or thermally polluted rivers emphasizes that water temperature is a crucial factor in the colonization of water distribution systems. *L. pneumophila* has been shown to be able to withstand temperatures of 50 °C for several hours, but does not multiply below 20 °C (Fliermans, Soracco & Pope, 1981; Katz & Hammond, 1987; Colbourne et al., 1988; Bentham 1993). It is for this reason that the recommended temperature for storage and distribution of cold water is below 25 °C and ideally below 20 °C. Thus, the presence of *Legionella* in an aquatic environment and warm temperatures are two factors that can increase the risk of Legionnaires' disease.

The presence of biofilms is important for *Legionella* survival and growth in water systems. Legionellae are found in sources such as distributed drinking-water supplies, which then feed into water systems within buildings and cooling towers, accounting for the bacteria's presence and subsequent growth in these artificial environments.

Chapter 3 discusses risk management of *Legionella*. The public health risk posed by legionellosis can be addressed by preventive measures — although the source of infection cannot be completely eradicated, risks can be substantially reduced. The preferred approach to health risk assessment in evaluating specific risks of exposure to *Legionella* from water systems is to develop a **water safety plan** (WSP), which provides a detailed and systematic assessment and prioritization of hazards, and operational monitoring of barriers and control measures. Chapter 3 outlines the process involved in developing a WSP to minimise proliferation of *Legionella* and exposure to the organism.

Chapters 4–8 are structured around the concept of a WSP. They are not intended to comprehensively address the WSP approach outlined in Chapter 3; rather, these chapters summarise the general principles and factors one would need to focus on in developing a WSP for the control of *Legionella* in the different environments and operating scenarios covered.

- **Potable water distribution systems** — Chapter 4 covers factors affecting microbial growth in potable water systems and in-building distribution systems. Distributed water is likely to contain some microorganisms, including legionellae. It is therefore reasonable to assume that all systems that use water could be seeded with microorganisms during construction, repair and maintenance, even if the water is treated. Risk factors that can promote the proliferation of legionellae include temperature, water quality, design, material used in construction and the presence of biofilms. The focus of attention in managing legionellae risks should be on preventing both **proliferation and exposure**. Therefore, Chapter 4 suggests control measures ranging from source water quality and treatment of source water to design of systems to prevent stagnation and control of temperature to minimise proliferation.
- **Cooling towers and evaporative condensers** — Cooling towers and evaporative condensers have historically been implicated in numerous outbreaks of Legionnaires' disease. Chapter 5 discusses the risk factors and management of cooling towers and evaporative condensers. Globally, the primary legionellae associated with outbreaks of disease from these systems appear to be *L. pneumophila* serogroup 1 MAb2 reactive strains. The major risk factor for legionellae proliferation appears to be neglect or insufficient maintenance. A significant proportion of outbreaks of Legionnaires' disease in these systems have been attributable to the **start-up of stagnant systems without adequate chemical treatment**. Cooling towers and evaporative condensers are generally designed to maximize operational performance of a thermal system; however, Chapter 5 spells out the importance of an effective water treatment programme in controlling legionellae proliferation. Such a programme has multiple benefits, in that it provides for more efficient operation from reduced fouling and a longer system life from reduced corrosion, while ensuring safer operation of the system due to reduced risk of legionellosis. Maintenance of properly treated cooling systems is also an essential element in reducing legionellae risks in these environments.

- *Health-care facilities* — Chapter 6 focuses on nosocomial cases of Legionnaires' disease, which tend to have a high case–fatality rate (the mortality rate can be as high as 40%), although they comprise a smaller proportion of reported cases of legionellosis than community-acquired cases. Underlying disease is a major risk factor for acquiring Legionnaires' disease. Initially, cooling towers were thought to be the main source of legionellae in health-care facilities, but many cases have been associated with piped hot and cold-water distribution systems. Maintenance of temperatures outside the 20–50 °C range in the network is the best way to prevent colonization of *Legionella* in distribution systems.
- *Hotels and ships* — Chapter 7 considers piped water systems of hotels, which are particularly susceptible to colonization by legionellae because of their large size, their complexity and their seasonal use patterns (which mean they may have long periods of stagnation and low use). Preventive and control measures follow the same procedures identified for other buildings; for example, they involve removing dead and blind ends, maintaining elevated temperatures in the hot-water system, and periodic disinfection and permanent chlorination of the cold-water system.

Chapter 7 also covers ships, which, like hotels, have complex water systems, and are difficult to link to outbreaks or cases because passengers have usually dispersed before developing symptoms. Ships also present particular challenges, as they are closed environments that may increase the opportunity for transmission of airborne infection. Hot and cold-water systems and spa pools have been implicated in a number of outbreaks of Legionnaires' disease on ships.

- *Natural spas, hot tubs and swimming pools* — Chapter 8 covers these devices. Although there are no recorded outbreaks of Legionnaires' disease associated with bathing in swimming pools, there is a risk of legionellosis from showers in the vicinity of pools, and these should be managed as for hot and cold distribution systems in public buildings.

Thermal water systems, including hot tubs and display spas, have been responsible for large outbreaks of Legionnaires' disease. Hot tubs are a particular risk, due to the warm water temperature (optimal for the growth of legionellae), high bather density, conditions that increase the risk of nutrients for bacterial growth, areas of pipework that do not receive disinfection from the pool water or hold stagnant water, and the potential to inhale aerosols at a short distance from the water surface. Design, installation, management and maintenance of these water systems must be undertaken with control of microbial growth in mind. Disinfection, cleaning, monitoring and regular service and maintenance are key factors in controlling *Legionella*.

Chapter 9 focuses on surveillance for Legionnaires' disease, which is now a statutory notifiable disease in most industrialized countries. National surveillance depends on the country's infrastructure and public health laws, and on surveillance principles and procedures. Because

of the impact Legionnaires' disease can have on tourism, the priority may be greater than local morbidity and mortality suggests. The chapter provides information on surveillance systems; it also gives guidance on policies and practice for outbreak management, and on institutional roles and responsibilities when an outbreak control team is convened.

Chapter 10 considers regulatory aspects of controlling *Legionella* in water systems and preventing legionellosis. Disease notification systems provide the basis for initiating investigations, identifying sources of infection, issuing public advice and limiting the scale and recurrence of outbreaks. Notification and investigation systems can be incorporated within regulations, which generally have a number of common features. The chapter also gives guidance on designing new regulations, emphasizing the key features that need to be considered, such as managerial responsibilities; registration and notification; system assessment and design; operational monitoring and verification; documentation of management plans and record keeping; and surveillance and audit. It also covers inclusion of specific regulations to deal with responses to outbreaks.

Chapter 11 covers laboratory aspects. Accurate diagnosis of *Legionella* is important, because timely and appropriate therapy is the key to improving patient outcomes. The chapter reviews the five methods currently used for the laboratory diagnosis of *Legionella* infections — isolation of the organism on culture media, paired serology, detection of antigens in urine, demonstration of the bacterium in tissue or body fluids using immunofluorescence microscopy, and detection of bacterial deoxyribonucleic acid (DNA) using the polymerase chain reaction.

Chapter 1 Legionellosis

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In 1976, an outbreak of severe pneumonia among the participants of the American Legion Convention in Philadelphia led to the description of Legionnaires' disease by Fraser et al. (1977). The disease was found to be caused by the bacterium *Legionella pneumophila* (*Legionella* after the legionnaires who were infected at the convention; *pneumophila* meaning "lung-loving"), belonging to the family Legionellaceae. The generic term "legionellosis" is now used to describe these bacterial infections, which can range in severity from a mild, febrile illness (Pontiac fever) to a rapid and potentially fatal pneumonia (Legionnaires' disease). *Legionella* has been retrospectively identified as the cause of outbreaks of Legionnaires' disease since 1947 (Terranova, Cohen & Fraser, 1978; McDade, Brenner & Bozeman, 1979).

Legionellosis emerged because of human alteration of the environment, since *Legionella* species are found in aquatic environments, and thrive in warm water and warm, damp places, such as cooling towers. Cases can be usefully grouped by the way in which they were acquired, as community acquired, domestically acquired, nosocomial (acquired in a health-care setting, or "health-care acquired") or travel associated.

This chapter describes:

- the characteristics of the main types of disease caused by *Legionella* (Section 1.1)
- the prevalence of *Legionella* and risk factors for disease (Section 1.2)
- treatment options (Section 1.3)
- the main types of organism causing legionellosis (Section 1.4)
- the factors affecting the pathogenicity of the causative organisms (their ability to cause disease) and their virulence (the degree of that ability, indicated by the mortality rate from the disease, or the organisms' ability to invade tissues) (Section 1.5).

1.1 Types of disease

This section describes the characteristics of Legionnaires' disease, Pontiac fever and extrapulmonary syndrome (caused when *L. pneumophila* spreads from the respiratory system to the body).

1.1.1 Legionnaires' disease

Symptoms

Legionnaires' disease lacks characteristic symptoms or signs — there is no typical syndrome, and not everyone exposed to the organism will develop symptoms of the disease (Yu et al., 1982; Macfarlane et al., 1984; Granados et al., 1989; Roig et al., 1991; Sopena et al., 1998; Ruiz et al., 1999; Gupta, Imperiale & Sarosi, 2001). However, several clinical signs are classically associated with Legionnaires' disease rather than with other causes of pneumonia. Table 1.1 (below) lists the most common symptoms of Legionnaires' disease and Pontiac fever.

Table 1.1 Main characteristics of Legionnaires' disease and Pontiac fever

Characteristic	Legionnaires' disease	Pontiac fever
Incubation period	2–10 days, rarely up to 20 days	5 hrs–3 days (most commonly 24–48 hrs)
Duration	Weeks	2–5 days
Case–fatality rate	Variable depending on susceptibility; in hospital patients, can reach 40–80%	No deaths
Attack rate	0.1–5% of the general population 0.4–14% in hospitals	Up to 95%
Symptoms	• Often non-specific • Loss of strength (asthenia) • High fever • Headache • Nonproductive, dry cough • Sometimes expectoration blood-streaked • Chills • Muscle pain • Difficulty in breathing, chest pain • Diarrhoea (25–50% of cases) • Vomiting, nausea (10–30% of cases) • Central nervous system manifestations, such as confusion and delirium (50% of cases) • Renal failure • Hyponatraemia (serum sodium <131 mmol/litre) • Lactate dehydrogenase levels >700 units/ml • Failure to respond to beta-lactam antibiotics or aminoglycosides • Gram stain of respiratory specimens with numerous neutrophils and no visible organisms	• Influenza-like illness (moderate to severe influenza) • Loss of strength (asthenia), tiredness • High fever and chills • Muscle pain (myalgia) • Headache • Joint pain (arthralgia) • Diarrhoea • Nausea, vomiting (in a small proportion of people) • Difficult breathing (dyspnoea) and dry cough

Sources: Woodhead & Macfarlane, 1987; Stout & Yu, 1997; Yu, 2000; Akbas & Yu, 2001; Mülazimoglu & Yu, 2001

Legionnaires' disease is often initially characterized by anorexia, malaise and lethargy; also, patients may develop a mild and unproductive cough. About half of patients develop pus-forming sputum, and about one third develop blood-streaked sputum or cough up blood (haemoptysis). Chest pain, whether pleuritic (i.e. involving infection of the lung lining) or non-pleuritic, is prominent in about 30% of patients, and may be mistaken for blood clots in the lungs when associated with haemoptysis. Gastrointestinal symptoms are prominent, with up to half of patients having watery diarrhoea, and 10–30% suffering nausea, vomiting and abdominal pains. Fever is present in almost all cases, and fever associated with chills usually develops within the first day (see references for Table 1.1).

Almost half of patients suffer from disorders related to the nervous system, such as confusion, delirium, depression, disorientation and hallucinations. These disorders may occur in the first week of the disease. Physical examination may reveal fine or coarse tremors of the extremities, hyperactive reflexes, absence of deep tendon reflexes, and signs of cerebral dysfunction. The clinical syndrome may be more subtle in immunocompromised patients.

Radiographic changes

The radiographic pattern of Legionnaires' disease is indistinguishable from that seen in other causes of pneumonia (Mülazimoglu & Yu, 2001). Radiological changes are visible from the third day after disease onset, usually beginning as an accumulation of fluid in part of the lung, which can progress to the other lobes, forming a mass or nodule. Diffuse accumulation of fluid occurs in the lungs of about one quarter of patients. Chest X-rays of immunocompromised patients receiving corticosteroids may show clearly defined areas of opacity around lung edges, which may be mistaken for pulmonary infarction. Abscesses can develop in immunosuppressed patients and, in rare cases, abscesses may penetrate the pleural space, causing pus formation (empyema) or a bronchopleural fistula (a hole between the bronchus and lung lining, allowing air to leak). Lung cavitation may occur up to 14 days after initial disease onset, even after appropriate antibiotic therapy and apparent clinical response. Pleural effusion (the collection of fluid inside the chest cavity around the lung) is reported in one third of legionellosis cases, and may occasionally precede the radiographical appearance of fluid accumulation within the lung.

Chest X-rays show progression of fluid accumulation, despite appropriate antibiotic therapy, in about 30% of cases; however, this does not necessarily indicate a progressive disease (Domingo et al., 1991). Instead, the spread indicates failure of treatment in association with simultaneous clinical deterioration.

Abnormalities may persist on X-ray for an unusually long time, even after the patient shows substantial clinical improvement; clearance rates of 60% at 12 weeks have been reported (Macfarlane et al., 1984; Stout & Yu, 1997; Yu, 2002).

Long-term effects

If untreated, Legionnaires' disease usually worsens during the first week and can be fatal. The most frequent complications are respiratory failure, shock and acute renal and multi-organ failure. Appropriate early treatment usually results in full recovery; however, long-term pathological conditions resulting from the disease (sequelae) may occur. Minor problems may include persistent pulmonary scars and restrictive pulmonary disease in some patients who experience severe respiratory failure. In severe infections, there are often general secondary symptoms, such as weakness, poor memory and fatigue, which can last for several months. Other neurological deficits that can arise from severe infection include residual cerebellar dysfunction (Baker, Farrell & Hutchinson, 1981), retrograde amnesia, and cerebellar signs and symptoms (Edelstein & Meyer, 1984), although retrograde amnesia is the only one of these deficits to be noted relatively frequently.

Incubation period

The incubation period is the time interval between initial exposure to infection and the appearance of the first symptom or sign of disease. The average incubation period of Legionnaires' disease is 2–10 days (WHO, 2004), although it may extend to even longer than 10 days. An epidemiological study of a major outbreak of Legionnaires' disease associated with a flower show in the Netherlands found that 16% of cases had incubation times longer than 10 days, with the average being 7 days (Den Boer et al., 2002; Lettinga et al., 2002).

Diagnosis and treatment

Attempts to establish predictive scores that identify *Legionella* pneumonia in individual patients have been unsuccessful. Although several clinical signs and symptoms have been described as characteristic of legionellosis (as outlined above), there is a considerable overlap of symptoms for Legionnaires' disease and *Legionella* pneumonia. This overlap makes it difficult to develop a checklist of characteristics for diagnosing individual patients infected with *Legionella* (Gupta, Imperiale & Sarosi, 2001; Mülazimoglu & Yu, 2001; Roig & Rello, 2003).

In targeting antibiotic therapy, it is best not to rely on diagnosis of a syndrome if there is no microbiological diagnosis. Generally, the recommended approach for all patients with pneumonia acquired in the community is an initial trial antimicrobial treatment, based on assessment of pneumonia severity and host-related risk factors (see Section 1.3).

Causative agents

Legionnaires' disease is usually caused by *L. pneumophila*, but in some cases one or more additional organisms may also be involved, resulting in a mixed (polymicrobial) infection. Culture of these co-infectors has revealed a wide spectrum of organisms, including aerobic bacteria (those that require free or dissolved oxygen, such as *Mycobacterium tuberculosis*), anaerobic bacteria (those from environments without such oxygen), viruses and fungi (Roig & Rello, 2003). Section 1.4 discusses the causative agents in more detail.

1.1.2 Pontiac fever

Symptoms

Pontiac fever is an acute, self-limiting, influenza-like illness without pneumonia (that is, it is “non-pneumonic”). Unlike Legionnaires’ disease, Pontiac fever has a high attack rate, affecting up to 95% of exposed individuals (Glick et al., 1978). The main symptoms are listed in Table 1.1.

Radiographic changes and long-term effects

Chest X-rays are normal, and recovery within one week is usual.

Incubation period

The incubation period is 24–48 hours.

Diagnosis and treatment

Treatment is supportive and aimed at relieving symptoms; complications rarely occur.

Causative agents

Depending on the causative agent, Pontiac fever may, in rare cases, not be as benign as previously thought (Jones et al., 2003). For example, Spieker et al. (1998) reported a case of acute disseminating encephalomyelitis that developed three weeks after a flu-like infection (Pontiac fever) with *L. cincinnatiensis*. Pontiac fever has also been associated with production of endotoxins (Fields et al., 2001).

Endotoxins can be extremely toxic to people, producing fever, shock and even death. It is not uncommon to find endotoxin associated with high heterotrophic plate counts (tests used to estimate the total number of all types of bacteria in an environmental sample). Therefore, further study is needed to establish whether endotoxin has a role in causing Pontiac fever where legionellae are also present. An outbreak in Scotland with Pontiac fever symptoms was caused by *L. micdadei*, and was named Lochgoilhead fever (Goldberg et al., 1989). Section 1.4 discusses the causative agents in more detail.

1.1.3 Extrapulmonary syndromes

It has been shown by autopsy that *L. pneumophila* can spread from the respiratory system to the body. Legionellae have been detected in the spleen, liver, kidney, myocardium, bone and bone marrow, joints, inguinal and intrathoracic lymph nodes and digestive tract (Lowry & Tompkins, 1993).

Table 1.2 provides details of cases of extrapulmonary syndromes associated with *Legionella* species.

Symptoms

The clinical manifestations of extrapulmonary *Legionella* infections are often dramatic. *Legionella* have been implicated in cases of sinusitis, cellulitis, pancreatitis, peritonitis and pyelonephritis, most often in immunocompromised patients (Eitrem, Forsgren & Nilsson, 1987; Stout & Yu, 1997). Lowry & Tompkins (1993) reported 13 extrapulmonary infections, including brain abscesses and sternal wound infections. The most commonly affected site is the heart (e.g. myocarditis, pericarditis, postcardiomyotomy syndrome and endocarditis) (Stout & Yu, 1997). Endocarditis due to *Legionella* spp. has been cited in only a few publications, and in all reported cases patients had a prosthetic valve (McCabe et al., 1984; Tompkins et al., 1988; Chen, Schapiro & Loutit, 1996). The patients showed low-grade fever, night sweats, weight loss, malaise, symptoms of congestive heart failure, and vegetation on echocardiography (Brouqui & Raoult, 2001). *Legionella* rarely spreads into the nervous system; more frequently, it leads to neurological manifestations of encephalomyelitis, cerebellum involvement and peripheral neuropathy (Shelburne, Kielhofner & Tiwari, 2004). *Legionella* meningoencephalitis may mimic the symptoms of herpes encephalitis (Karim, Ahmed & Rossoff, 2002).

Diagnosis

Legionellosis should be considered in the differential diagnosis of patients showing a combination of neurological, cardiac and gastrointestinal symptoms, particularly in the presence of radiographic pneumonia (Shelburne, Kielhofner & Tiwari, 2004).

Causative agent

Among the four species of *Legionella* responsible for extrapulmonary infections, *L. pneumophila* was the most commonly isolated bacteria (Lowry & Tompkins, 1993).

Table 1.2 Extrapulmonary infections caused by Legionella species

Number of cases	Age (years)	Site of infection	Legionella species	No-socomial	Transmission
1	40	Maxillary sinus	<i>L. pneumophila</i> sg 1	No	Unknown
1	62	Cutaneous abscess	<i>L. micdadei</i>	No	Seeding from pneumonia
1	33	Brain abscess	<i>L. jordanis</i>	No	Unknown
1	70	Bowel, liver, kidney, spleen, peritonitis	<i>L. pneumophila</i> sg 1	No	Possible oral ingestion
1	71	Hip wound	<i>L. pneumophila</i> sg 4	Yes	Water contact
1	51	Myocarditis	<i>Legionella</i> (not speciated)	No	Unknown
1	22	Pericardial effusion	<i>Legionella</i> (not speciated)	No	Unknown
1	43	Pericardial effusion	<i>L. pneumophila</i> sg 3	No	Seeding from pneumonia
1	33	Pericardial effusion	<i>L. pneumophila</i> not serotyped)	No	Seeding from pneumonia
1	27	Bovine A-V-fistula infection	<i>L. pneumophila</i> sg 1	No	Seeding from pneumonia
1	69	Synthetic A-V-fistula infection	<i>L. pneumophila</i> sg 1	No	Seeding from pneumonia
1	62	Acute pyelonephritis with abscess	<i>L. pneumophila</i> sg 4	Yes	Seeding from pneumonia
1	46	Perirectal abscess	<i>L. pneumophila</i> sg 3	Yes	Water contact
7	51 (mean)	Prosthetic valve endocarditis	<i>L. pneumophila</i> sg 1 (2 strains) and <i>L. dumoffii</i>	Yes	Unknown
3	3 weeks, 27, 85	Sternal wound infection	<i>L. pneumophila</i> sg 1 and <i>L. dumoffii</i>	Yes	Water contact

sg = serogroup

Source: Reprinted from Lowry & Tompkins, 1993, with permission from the Association for Professionals in Infection Control & Epidemiology, Inc.

1.2 Prevalence and risk factors

The exact incidence of legionellosis worldwide is unknown, because countries differ greatly in the methods they use for ascertaining whether someone has the infection and in reporting of cases. Also, the reported incidence of Legionnaires' disease varies widely according to the intensity of investigation and the diagnostic methodology applied (as discussed in Chapter 9). Table 1.3 provides some useful definitions for epidemiological monitoring, used throughout this publication. Table 1.4 shows European cases, by category, from 1994 to 2004.

Table 1.3 Useful definitions for epidemiological monitoring

Legionnaires' disease	Case definitions
Confirmed cases ^a	Clinical or radiological evidence of pneumonia and a microbiological diagnosis by culture of the organism from respiratory specimens, or a fourfold rise in serum antibody ^b levels against <i>L. pneumophila</i> serogroup ^c (sg) 1, or detection of <i>L. pneumophila</i> antigen ^d in urine or positive direct immunofluorescence assay (DFA) test.
Presumptive cases	Clinical or radiological evidence of pneumonia and a microbiological diagnosis of a single high antibody level against <i>L. pneumophila</i> sg 1 or a seroconversion ^e demonstrated against <i>Legionella</i> species and serogroups other than <i>L. pneumophila</i> sg 1.
Health-care acquired (nosocomial) cases	Depending on length of stay in hospital before onset and environmental investigation results, cases are definitely, probably or possibly nosocomial (see Box 1.1, below, for details of this classification).
Travel-associated cases ^f	Case associated with one or more overnight stays away from home, either in the country of residence or abroad, in the 10 days before onset of illness.
Travel-associated clusters ^g	Two or more cases stayed at the same accommodation, with onset of illness within the same two years (Lever & Joseph, 2003).
Community clusters ^h	Two or more cases linked by area of residence or work, or places visited, and sufficient closeness in dates of onset of illness to warrant further investigation.
Community outbreaks ⁱ	Community clusters for which there is strong epidemiological evidence of a common source of infection, with or without microbiological evidence, and in response to which control measures have been applied to suspected sources of infection.
Domestically acquired cases	Depending on the elimination of all other sources of exposure, and the case being known to have used the domestic water system during the incubation period, and environmental and clinical results positive for <i>Legionella</i> , cases may be suspected, probably or definitely domestically acquired.

^a When submitted to a *Legionella* reference laboratory, it is recommended that all positive serum specimens are examined by the indirect fluorescent antibody test (Boswell, Marshall & Kudesia, 1996) in the presence of campylobacter blocking fluid, to eliminate cross-reactions between organisms.

- ^b Proteins produced by the body's immune system that recognize and help fight infections and other foreign substances in the body.
- ^c A serogroup is a subdivision of a species or subspecies distinguishable from other strains on the basis of antigenic character testing for recognizable antigens on the surface of the microorganism.
- ^d Antigens are foreign substances that stimulate the production of antibodies by the immune system.
- ^e Seroconversion is the development of antibodies in blood serum as a result of infection or immunization.
- ^f Cases of legionellosis acquired during travel (e.g. from a cruise ship or a hotel).
- ^g European Working Group for Legionella Infections (EWGLI)¹ definition, introduced in January 2001.
- ^h This is a working definition: the decision to follow up cases will be made locally or nationally.
- ⁱ Community clusters or community-acquired cases are those that are not travel-acquired, health-care acquired or domestically acquired (i.e. acquired in the patient's home).

Table 1.4 Category of European cases, 1994–2004

Category of cases	Year										
	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Nosocomial	151	157	105	215	181	195	275	333	277	347	309
Community	186	270	617	388	478	679	659	1475	1767	2106	1884
Travel associated	190	194	246	290	297	439	500	674	944	927	984
Not known	634	634	595	451	486	823	722	988	1691	1072	1369
Total	1161	1255	1563	1344	1442	2136	2156	3470	4679	4452	4546

Source: Information obtained from the European Working Group for Legionella Infections (EWGLI)²

1.2.1 Community-acquired pneumonia

The term “community-acquired pneumonia” (CAP) refers to cases that are not acquired through travel, health care or the domestic setting. CAPs have a high rate of hospital admission, with less than 1% being managed at home. Legionnaires’ disease can account for up to 30% of CAPs requiring admission to intensive care (Woodhead et al., 1987; Macfarlane et al., 1993). In recent studies involving hospitalized patients with CAP in the United States of America (USA), Europe, Israel and Australia, 0.5–10% had Legionnaires’ disease, with an average level of about 2% (NHMRC, 1988; Fang et al., 1990; Rello et al., 1993; Mundy et al., 1995; Olacchia et al., 1996; Marston et al., 1997; Stout and Yu, 1997; Boldur et al., 1999; Cosentini et al., 2001; Lim et al., 2001; Fields, Benson & Besser, 2002; Mandell et al., 2002; Mandell et al., 2004; Edelstein & Cinaciotto, 2005). The proportion of CAPs resulting in severe pneumonia

1 <http://www.ewgli.org/>

2 <http://www.ewgli.org/>

is higher for Legionnaires' disease than for other causes; consequently, there is a higher mortality rate (Ewig & Torres, 1999).

1.2.2 Nosocomial infections

Box 1.1 gives details of the classifications used for nosocomial Legionnaires' disease.

Box 1.1 Classifications of nosocomial Legionnaires' disease

- **Definite nosocomial** — Legionnaires' disease in a person who was in hospital for 10 days before the onset of symptoms.
- **Probable nosocomial** — Legionnaires' disease in a person who was in hospital 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 (by monoclonal antibody subgrouping or by molecular typing methods) from isolates obtained from the hospital water system at about the same time.
- **Possible nosocomial** — Legionnaires' disease in a person who was in hospital 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 (or the residential institution).

Source: Lee & Joseph (2002)

Risk factors for nosocomial pneumonia are:

- recent surgery
- intubation (insertion of a tube into the trachea to assist breathing) and mechanical ventilation
- aspiration (the presence of foreign matter, such as food or nasogastric tubes, in the lung)
- use of respiratory therapy equipment.

Aspiration may occur in patients with immunosuppression or swallowing disorders (e.g. after an operation on the neck) (Stout & Yu, 1997). Nasogastric tubes have been identified as risk factors in several studies of nosocomial legionellosis, with microaspiration of contaminated water presumed to be the mode of entry (Marrie et al., 1991; Blatt et al., 1994; Stout & Yu, 1997).

Patients suffering from Legionnaires' disease are significantly more likely to have undergone endotracheal tube placement or to have been intubated for longer than patients with other types of pneumonia (Muder et al., 1983; Strebel et al., 1988; Kool et al., 1998). However, a recent study failed to detect colonization of the oesophageal tract by *Legionella* (Pedro-Botet et al., 2002).

Wound infection may be caused by direct entry of legionellae into damaged skin, and has been observed after immersion of a wound in contaminated water (Brabender et al., 1983; Lowry et al., 1991). However, there is no evidence to support pulmonary disease arising from wound infection. Although cases of infection have been reported among pregnant women (which could increase their risk of premature labour), pregnancy is not considered a risk factor for legionellosis (Roig & Rello, 2003). The most susceptible hosts are immunocompromised patients, including solid-organ transplant recipients and those receiving corticosteroid treatments (Arnow et al., 1982; Strebel et al., 1988).

Tables 1.5 and 1.6 identify the risk factors for *Legionella* infection.

Table 1.5 Risk factors for *Legionella* infection, by category

	Community acquired	Travel associated	Nosocomial
Modes of transmission	Inhalation of contaminated aerosol ^a	Inhalation of contaminated aerosol	Inhalation of contaminated aerosol, aspiration, wound infection
Sources of <i>Legionella</i>	Cooling towers; hot and cold-water systems; spa pools, thermal pools, springs; humidifiers; domestic plumbing; potting mixes and compost	Cooling towers; hot and cold-water systems; spa pools, thermal springs and pools; humidifiers	Cooling towers; hot and cold-water systems; spa pools, natural pools, thermal springs; respiratory therapy equipment; medical treatment
Reservoir of <i>Legionella</i>	Industrial sites, shopping centres, restaurants, clubs, leisure centres, sports clubs, private residences	Hotels, cruise ships, camp sites, shopping centres, restaurants, clubs, leisure centres, sports clubs	Hospitals, medical equipment
Risk factors (environmental)	Proximity to sources of transmission, poor design or poor maintenance of cooling water systems, inadequate staff training	Stay in accommodation designed for short stays and seasonal use; intermittent room occupancy and water use; intermittent water supply and fluctuating water temperature control; complex water systems; lack of trained staff to manage water systems	Complex water distribution system, long pipe runs, poor water temperature control, low water flow rates
Risk factors (personal)	Age >40 years; male; underlying disease such as diabetes; chronic heart disease; smoking; immunosuppression (especially with glucocorticosteroids and chronic debilitating illness); structural pulmonary comorbidity ^b ; chronic renal failure; recent travel; haematological malignancy; iron overload; other immunosuppression	Age >40 years; male; heavy smoking, alcohol abuse; change in lifestyle; underlying disease such as diabetes; chronic heart disease, other immunosuppression	Age >25 years; transplant patient; other immunosuppression; surgery, especially head and neck; cancer, including leukaemias/lymphomas; diabetes; treatment with respiratory devices; chronic heart/lung disease; smoking, alcohol abuse

^a A suspension of fine solid or liquid particles in a gas, such as air

^b A disease or disorder that is not directly caused by another disorder but occurs at the same time

Sources: Fields, Benson & Besser, 2002; Joseph, 2004a; Marston, Lipman & Brieman, 1994; Ricketts & Joseph, 2004; Vikram & Bia, 2002

Table 1.6 Risk factors for *Legionella* infection, by reservoir

	Cooling water systems	Hot and cold-water systems	Hot tubs Natural spa pools Thermal springs	Humidifiers Respiratory equipment	Potting mixes Compost
Commonly implicated <i>Legionella</i> species	Predominantly <i>L. pneumophila</i> sg 1	<i>L. pneumophila</i> sg 1, 2, 4, 6, 12, <i>L. micdadei</i> , <i>L. bozemanii</i> , <i>L. feeleii</i> and others	<i>L. pneumophila</i> sg 1, <i>L. micdadei</i> , <i>L. gormanii</i> , <i>L. anisa</i>	<i>L. pneumophila</i> sg 1, 3, and others,	Exclusively <i>L. longbeachae</i>
Modes of transmission	Inhalation of aerosol	Inhalation of aerosol, aspiration	Inhalation of aerosol, possible aspiration	Inhalation of aerosol	Not known
Disease outbreaks	Rapid onset over wide area, resolve within incubation period	Low numbers of cases over prolonged periods	Rapid onset confined to users and those in close proximity	Low numbers over prolonged periods. Rapid onset confined to users and those in close proximity	Low numbers of cases over prolonged periods
Risk factors (environmental)	Proximity of population, seasonal/ climatic conditions, intermittent use, poor maintenance, poor design	Complex water systems, long pipe runs, poor temperature control, low flow rates/ stagnation	Poor maintenance, stagnant areas in system	Use of non-sterile water, poor maintenance/cleaning, operation at temperatures conducive to <i>Legionella</i> growth	Seasonal (spring and autumn), use of potting mixes/compost, gardening

sg = serogroup

1.2.3 Sporadic cases of pneumonia

Sporadic cases are isolated or unique cases. Severe *Legionella* infections have occurred among previously healthy people, including young people without underlying disease, and those without other known risk factors (Falguera et al., 2001). The role of *Legionella* in causing an acute increase in the severity of symptoms of chronic obstructive pulmonary disease is unclear (Ewig, 2002).

Taking France as an example, 807 cases of Legionnaires' disease were notified by the French National Public Health Centre in 2001. In 558 of these cases, predisposing factors included:

- cancer or blood disease (11%)
- immunosuppressant treatment (12%)
- diabetes (10%)
- smoking (40%).

In 2001, 14% of the cases (105 cases) stayed in a hospital or a clinic during the incubation period, compared with 20% in 2000. An exposure to risk within the 10 days before the onset of the disease was reported for 335 patients (42%) (see Table 1.7).

Table 1.7 Risk exposures among Legionnaires' disease declared cases in France, 1999–2002

Risk exposures	1999		2000		2001		2002	
	<i>n</i> = 440	%	<i>n</i> = 610	%	<i>n</i> = 807	%	<i>n</i> = 1021	%
Hospital	73	17	119	20	105	14	100	10
Hotel/camp site	46	10	54	9	88	11	118	12
Thermal cure	7	1	6	1	7	1	9	1
Other health institutes	5	1	6	1	9	1	6	<1
Travel	22	5	17	3	30	4	21	2
Temporary residence	–	–	–	–	27	3	29	3
Retirement homes	–	–	–	–	18	2	35	3
Work	–	–	–	–	28	4	34	3
Other	49	11	91	15	23	3	85	8
Total	202	45	293	49	335	42	438	43

Source: Campese et al., 2002 (Reproduced with permission of the Institute de Veille Sanitaire, France)

1.2.4 Rates of mortality and survival

The case–fatality rate depends on the severity of disease, how it was acquired, timely determination through diagnostic methods of whether or not a person is infected with the disease (ascertainment of infection), the appropriateness and timing of initial antimicrobial treatment, and other risk factors present (Tkatch et al., 1998; Fernandez et al., 2002; Garcia-Fulgueiras et al., 2003; Roson et al., 2004; Edelstein & Cianciotto, 2005).

In the outbreak of Legionnaires' disease in Philadelphia in 1976, 34 out of 182 patients (18.7%) died (Fraser et al., 1977). Subsequently, average mortality has been confirmed to be about 15–20% of hospitalized cases (Edelstein & Meyer, 1984; Guerin, 1992; Roig & Rello, 2003). In the USA, the case–fatality rate was recorded as up to 40% in nosocomial cases, compared with 20% among people with community-acquired legionellosis (CDC, 1997a). More recent data from the USA and Australia showed case–fatality rates of 14% for nosocomial infections and 5–10% for community-acquired infections (Benin et al., 2002; Howden et al., 2003). In Europe, the overall case–fatality rate is about 12%.³

Early ascertainment is an important factor for patient survival. In the largest recorded outbreak, which occurred in Murcia, Spain, there were 449 confirmed cases, but the case–fatality rate was only 1% (Garcia-Fulgueiras et al., 2003). This low fatality rate was probably due to the clinicians' awareness of legionellosis risk, as well as recognition that survival and recovery depend on timely intervention and the correct choice of antimicrobial therapy, particularly in severe cases (Tkatch et al., 1998; Gacouin et al., 2002; Roig & Rello, 2003).

Advanced age and comorbidity are predictors of death by Legionnaires' disease. One study evaluated prognostic factors of severe *Legionella* pneumonia cases admitted to an intensive care unit (el Ebiary et al., 1997). In that study, the only independent factor related to death was an APACHE score greater than 15 at admission (APACHE — acute physiology and chronic health evaluation — is an algorithm for predicting hospital mortality). Cunha (1998) has also published a scoring system, based on clinical signs of Legionnaires' disease and laboratory abnormalities.

1.3 Treatment of Legionnaires' disease

Diagnostic tests

Tests for Legionnaires' disease should ideally be performed for all patients with pneumonia at risk, including those who are seriously ill, whether or not they have clinical features suggesting legionellosis. Tests for Legionnaires' disease should also be performed for patients displaying symptoms that do not match any other diagnosis, and particularly on ill patients who are older than 40 years, immunosuppressed, or unresponsive to beta-lactam antibiotics, or who might have been exposed to *Legionella* during an outbreak (Bartlett et al., 1998). Urine antigen tests, and cultures of sputum or bronchoalveolar lavage (washing the bronchial tubes and alveoli with repeated injections of water), are the most suitable clinical tests for *Legionella*. Chapter 11 discusses diagnostic laboratory tests for *Legionella*.

³ <http://www.ewgli.org/>

Evaluation of antimicrobial agents

When extracellular, legionellae are susceptible to a wide range of antimicrobial agents. However, in an infection, where the microorganism is inside the cell; the only antimicrobial agents that are clinically useful are those that achieve high intracellular concentrations. Therefore, new drugs have to be evaluated in regard to:

- their minimum inhibitory concentration values against *Legionella* spp.
- their activity in cellular culture systems
- their activity in animal studies
- the clinical context.

Suggested treatments

Only a few small, controlled clinical studies of antibiotic treatment for Legionnaires' disease have been completed; hence, the evidence for treatment recommendations is limited (Thornsberry, Baker & Kirven, 1978; Yoo et al., 2004; Yu et al., 2004). One small clinical study showed that treatment with fluoroquinolone pefloxacin gives patients a higher survival rate than treatment with erythromycin (Dournon et al., 1990).

The new macrolide antibiotics, such as clarithromycin and azithromycin, show more effective in-vitro activity and a better intracellular and tissue penetration than erythromycin, as do the quinolones. Beta-lactam antibiotics are not effective against Legionnaires' disease, but are the first choice of antibiotics for pneumococcal pneumonia, and are used together with macrolides to treat severe pneumonia. Where a rapid diagnostic test for Legionnaires' disease is not in use, many people presenting with this disease are simply treated with macrolides plus beta-lactam antibiotics, because a delay in the application of appropriate therapy for *Legionella* pneumonia significantly increases mortality (Stout & Yu, 1997).

Table 1.8 lists the various treatments for different groups of patients with Legionnaires' disease.

Table 1.8 Potential treatments for different patient groups

Treatment	Patient group	Reference	Comments
Respiratory fluoroquinolone	New transplant recipients		Generally recommended, because of the pharmacological interaction of the macrolides and rifampicin with immunosuppressive medication, and with each other
Rifampicin with erythromycin, clarithromycin, or a tetracycline	Severely ill patients	Vesley, Pien & Pien (1998)	No clinical evidence
Highly active fluoroquinolone (e.g. levofloxacin, ciprofloxacin, moxifloxacin, and probably gatifloxacin) or azithromycin	Severely ill patients	Ewig, Tuschy & Fatkenheuer (2002)	Removes need for rifampicin therapy
Imipenem, clindamycin, and trimethoprim-sulfamethoxazole	General use	Stout & Yu (1997)	Have been used for treatment with mixed success; their use for treatment of Legionnaires' disease is not reliable
Broad-spectrum antibiotic	Patients with mild Legionnaires' disease	Edelstein (1994); Beovic et al. (2003)	
Intravenous azithromycin or a respiratory quinolone; or doxycycline (200 mg twice a day)	Patients who are immunocompromised or have a potentially life-threatening infection	Muder (2005); Tompkins et al. (1988); Brouqui & Raoult (2001)	
Erythromycin	Patients with extra-pulmonary legionellosis	Lowry & Tompkins (1993); Park, Pugliese & Cunha (1994); Brouqui & Raoult (2001)	Drainage of purulent material or surgical intervention also commonly needed

Parenteral therapy is usually given at least until there is a clinical response, although outpatients with mild disease do well with oral therapy. Most patients recover from fever within 3–5 days. Total duration of treatment is normally 10–14 days (5–10 days for azithromycin), but a 21-day course has been recommended for immunosuppressed patients, as well as for those with severe disease (e.g. extensive evidence of disease on chest radiographs). However, chest X-rays are not effective for monitoring the success of the therapy (see Section 1.1.1).

Adverse effects

The principal adverse effects of a macrolide treatment include gastrointestinal symptoms, such as diarrhoea and vomiting, and effects on the ears (when given in high doses). Relatively frequently, the respiratory fluoroquinolones cause adverse effects such as gastrointestinal symptoms and central nervous system disturbances. Alteration to the electrocardiogram (ECG or EKG) (i.e. prolongation of the QT interval — the duration of the contraction of the heart's main chambers) precludes the use of these quinolones in patients with severe electrolyte imbalances, irregular heartbeats or severe congestive heart failure. Erythromycin has also been reported to cause ventricular fibrillation (disorganized twitching of the heart muscle) and QT prolongation, and should be used with caution in patients with heart disease, especially when the drug is rapidly administered into the bloodstream via a central venous catheter.

1.4 Types of organism causing disease

1.4.1 Taxonomy

Since Legionnaires' disease was recognized, characterization of the strains isolated from patients has led to the creation of a new bacterial genus, *Legionella*, belonging to the family Legionellaceae (Brenner, Steigerwalt & McDade, 1979). Some investigators (Garrity, Brown & Vickers, 1980; Brown, Garrity & Vickers, 1981) have proposed placing the legionellae in three separate genera — *Legionella*, *Fluoribacter* and *Tatlockia* — on the basis of low DNA (deoxyribonucleic acid) hybridization values between some *Legionella* species (Fox & Brown, 1993). However, other studies have shown that the family Legionellaceae forms a single subgroup, sharing a common ancestor within the gamma-s subdivision of the Proteobacteria. Data using 16S ribosomal RNA (ribonucleic acid) analysis support a single family, showing that all legionellae studied are more than 95% related (Fry et al., 1991).

Within the genus *Legionella*, the DNA relatedness between strains of a given species is unusually high (>90%), whereas DNA relatedness between one species and another is less than 70% (Brenner, 1986). Many definitions for bacterial genera and species have been suggested; however, it is likely that the integrated use of phylogenetic and phenotypic characters is necessary for the delineation of bacterial taxa at all levels (Murray et al, 1990). The nearest genetic relative to Legionellaceae is *Coxiella burnetii* (Marti, Garcia & Bustillo, 1990), the cause of Q fever. The Legionellaceae and *C. burnetii* have similar intracellular lifestyles, and may have common genes associated with the infection processes in their hosts.

1.4.2 Species and serogroups associated with disease

Number of species and serogroups

The “type” or representative species of *Legionella* is *L. pneumophila*, because it was the first species to be described. The number of species, subspecies and serogroups of legionellae continues to increase. Although *L. pneumophila* causes most cases of Legionnaires’ disease, other species can also cause the disease, particularly in nosocomial cases. The genus *Legionella* currently has at least 50 species comprising 70 distinct serogroups. These serogroups and their clinical manifestations are shown in Table 1.9 (Drozanski, 1991; Hookey et al., 1996; Riffard et al., 1996; Fry & Harrison, 1998; Fields, Benson & Besser, 2002; La Scola et al., 2004). There are 16 serogroups of *L. pneumophila* — two each in *L. bozemanii*, *L. longbeachae*, *L. feeleii*, *L. hackeliae*, *L. sainthelensi*, *L. spiritensis*, *L. erythra*, and *L. quinlivanii*, and a single serogroup in each of the remaining species.

Table 1.9 *Legionella* species and serogroups

<i>Legionella</i> species	Sero-groups	Association with clinical cases	Reference
<i>L. adalaidensis</i>		Unknown	Benson et al., 1996a; Benson & Fields, 1998
<i>L. anisa</i>		Yes	Bornstein et al., 1989a; Fenstersheib et al., 1990; Thacker et al., 1990
<i>L. beliardensis</i>		Unknown	Lo Presti et al., 2001
<i>L. birminghamensis</i>		Yes	Wilkinson et al., 1987;
<i>L. bozemanii</i>	2	Yes	Boldur et al., 1985; Bornstein et al., 1987; Bazovska & Spalekova, 1994
<i>L. brunensis</i>		Unknown	Wilkinson et al., 1988
<i>L. busanensis</i>		Unknown	Park et al., 2003
<i>L. cherrii</i>		Unknown	Brenner et al., 1985; Edelstein & Edelstein, 1989
<i>L. cincinnatiensis</i>		Yes	Thacker et al., 1988a; Jernigan et al., 1994; Spieker et al., 1998
<i>L. drozanskii</i>		Unknown	Adeleke et al., 2001
<i>L. dumoffii</i>		Yes	Edelstein & Pryor, 1985; Fang, Yu & Vickers, 1989
<i>L. drancourtii</i>		Unknown	La Scola et al., 2004
<i>L. erythra</i>	2	Yes	Brenner et al., 1985; Saunders, Doshi & Harrison, 1992; Fields, Benson & Besser, 2002
<i>L. fairfieldensis</i>		Unknown	Thacker et al., 1991
<i>L. fallonii</i>		Unknown	Adeleke et al., 2001
<i>L. feeleii</i>		Yes	Herwaldt et al., 1984
<i>L. geestiana</i>		Unknown	Dennis et al., 1993
<i>L. genomospecies 1</i>		Unknown	Benson et al., 1996b
<i>L. gormanii</i>		Yes	Lode et al., 1987; Griffith et al., 1988

Legionella species	Sero-groups	Association with clinical cases	Reference
<i>L. gratiana</i>		Unknown	Bornstein et al., 1989b
<i>L. gresilensis</i>		Unknown	Lo Presti et al., 2001
<i>L. hackeliae</i>	2	Yes	Wilkinson et al., 1985; Brenner et al., 1985
<i>L. israelensis</i>		Unknown	Bercovier et al., 1986; Sonesson et al., 1994
<i>L. jamestowniensis</i>		Unknown	Wilkinson et al., 1990; Brenner et al., 1985
<i>L. jordanis</i>		Yes	Cherry et al., 1982; Thacker et al., 1988b
<i>L. lansingensis</i>		Yes	Thacker et al., 1992
<i>L. londiniensis</i>	2	Unknown	Dennis et al., 1993
<i>L. longbeachae</i>	2	Yes	McKinney et al., 1981; Boldur et al., 1985; Chereshtsky & Bettelheim, 1986; Eitrem, Forsgren & Nilsson, 1987; Lode et al., 1987
<i>L. lytica (comb. nov.)</i>		Unknown	Birtles et al., 1996
<i>L. maceachernii</i>		Yes	Brenner et al., 1985; Merrell et al., 1991
<i>L. micdadei</i>		Yes	Hebert et al., 1980
<i>L. moravica</i>		Unknown	Wilkinson et al., 1988
<i>L. nautarum</i>		Unknown	Dennis et al., 1993
<i>L. oakridgensis</i>		Yes	Orrison et al., 1983; Tang, Toma & MacMillan, 1985
<i>L. parisiensis</i>		Yes	Lo Presti et al., 1997
<i>L. pneumophila</i>	16	Yes	Brenner et al., 1985; Yu, 2000
<i>L. quateirensis</i>		Unknown	Dennis et al., 1993
<i>L. quinlivanii</i>	2	Unknown	Benson et al., 1989; Birtles et al., 1991; Wilkinson et al., 1990
<i>L. rowbothamii</i>		Unknown	Adeleke et al., 2001
<i>L. rubrilucens</i>		Unknown	Brenner et al., 1985; Saunders, Doshi & Harrison, 1992
<i>L. sainthelensi</i>	2	Yes	Benson et al., 1990
<i>L. santacrucis</i>		Unknown	Brenner et al., 1985; Lee et al., 1993
<i>L. shakespearei</i>		Unknown	Verma et al., 1992
<i>L. spiritensis</i>	2	Unknown	Brenner et al., 1985; Harrison et al., 1988
<i>L. steigerwaltii</i>		Unknown	Brenner et al., 1985; Edelstein & Edelstein, 1989
<i>L. taurinensis</i>		Unknown	Lo Presti et al., 1999
<i>L. tusconensis</i>		Yes	Thacker et al., 1989
<i>L. wadsworthii</i>		Yes	Edelstein, 1982a
<i>L. waltersii</i>		Unknown	Benson et al., 1996b
<i>L. worsleiensis</i>		Unknown	Dennis et al., 1993

Other causes of infection

In Europe, approximately 70% of *Legionella* infections are caused by *L. pneumophila* serogroup 1, 20–30% are caused by other serogroups, and 5–10% are caused by non-pneumophila species (Joseph, 2002a).

The majority of human infections with species other than *L. pneumophila* are pneumonic, and occur after exposure to *Legionella* (Fang, Yu & Vickers, 1989). Of the reported non-pneumophila infections, the causes of infection are (Reingold et al., 1984; Fang, Yu & Vickers, 1989):

- *L. micdadei* (60%)
- *L. bozemanii* (15%)
- *L. dumoffii* (10%)
- *L. longbeachae* (5%)
- other species (10%).

L. longbeachae has been associated with exposure to potting composts in Australia, the USA and Japan (Steele, Lanser & Sangster, 1990; Steele, Moore & Sangster, 1990). The mechanisms of infection from potting compost are not fully understood. Outbreaks of legionellosis associated with construction or maintenance are likely to be the result of sloughing of biofilms (the slimy matrices produced and inhabited by bacteria, which enable them to adhere to a surface) or descaling of plumbing systems caused by changes in water flow or pressure (Storey, Ashbolt & Stenstrom, 2004b; see Chapter 2 for more information). Recently, it has been suggested that there may be a spectrum of illness from a single source, with several reports of outbreaks involving cases of both Legionnaires' disease and Pontiac fever.

Under appropriate conditions, most *Legionella* that can grow at body temperatures may be able to cause human infections (Fields, 1996). Infections due to species other than *L. pneumophila* may be underdetected, because of a lack of appropriate diagnostic tests (Fields, Benson & Besser, 2002).

The *Legionella* count alone cannot be used to predict whether a source positive for the bacterium will cause infection. The likelihood that a source will cause an infection depends on several factors: the load of bacteria, the effectiveness of dissemination, the way in which the bacteria multiply and the source's ability to form aerosols.

Legionella-like amoebal pathogens

Some legionellae cannot be grown on routine *Legionella* culture media, and have been termed *Legionella*-like amoebal pathogens (LLAPs) (see Chapter 11). These organisms have been isolated and maintained by co-cultivating the bacteria with their protozoan hosts. One LLAP strain was isolated from the sputum of a pneumonia patient by enrichment in amoebae and is considered to be a human pathogen (Fry et al., 1999; Marrie et al., 2001). Additional LLAP strains may be human pathogens, but proving this is difficult, because they cannot be detected by conventional techniques used for legionellae. Recently, three LLAP strains were named *Legionella* species (Adeleke et al., 2001; La Scola et al., 2004).

Typing

Helbig et al. (1995) suggested that differences in the virulence of *Legionella* species or serogroups are associated with different epitopes within the bacterial cell wall (epitopes are parts of a foreign organism or its proteins that are recognized by the immune system and targeted by antibodies, cytotoxic T cells or both). Tests using monoclonal subtyping show that the strains of *L. pneumophila* serogroup 1 most commonly associated with disease in humans share a common epitope (Watkins et al., 1985; Ehret, von Specht & Ruckdeschel, 1986; Dournon et al., 1988). Depending on the typing scheme used, these strains may be referred to as Pontiac (Watkins et al., 1985); monoclonal antibody (MAb) 2-reactive (Joly et al., 1986) or MAb 3/1-positive (Dresden Panel, 2002; Helbig et al., 2002).

In a European-wide study of *L. pneumophila*, 1335 cases of Legionnaires' disease were serotyped, and monoclonal types of serogroup 1 were grouped according to the presence of the epitope recognized by MAb 3/1 (Dresden Panel, 2002). Approximately 66.8% of cases were MAb 3/1-positive, and 11.7% of the overall isolates belonged to the MAb 3/1-negative serogroup 1 subgroups. Monoclonal subtype Philadelphia was the most frequently recognized. Most of the MAb 3/1-negative strains were from nosocomial infections (53.5%), with 27.3% from community-acquired cases and 14.2% from travel-associated cases (Helbig et al., 2002). The proportion of MAb 3/1-negative strains was significantly higher in the Scandinavian region than in Mediterranean countries or the United Kingdom, for both community-acquired and nosocomial cases.

1.5 Virulence and pathogenicity

Various studies have shown that the pathogenesis and ecology of *Legionella* are inherently related. Rowbotham first demonstrated that *L. pneumophila* could infect amoeba, and characterized the life cycle of *Legionella* in amoeba (Rowbotham, 1980). Horwitz's classical experiments demonstrated that *L. pneumophila* multiplied intracellularly in human macrophages by avoiding phagosome–lysosome fusion (Horwitz, 1983). There are striking similarities in the processes by which legionella infect protozoa and mammalian phagocytic cells (Bozue & Johnson 1996; Horwitz 1984; Garduno et al., 2002). The abilities of *Legionella* to infect mammalian and protozoan cells are related, using common genes and gene products.

1.5.1 Overview and life-cycle

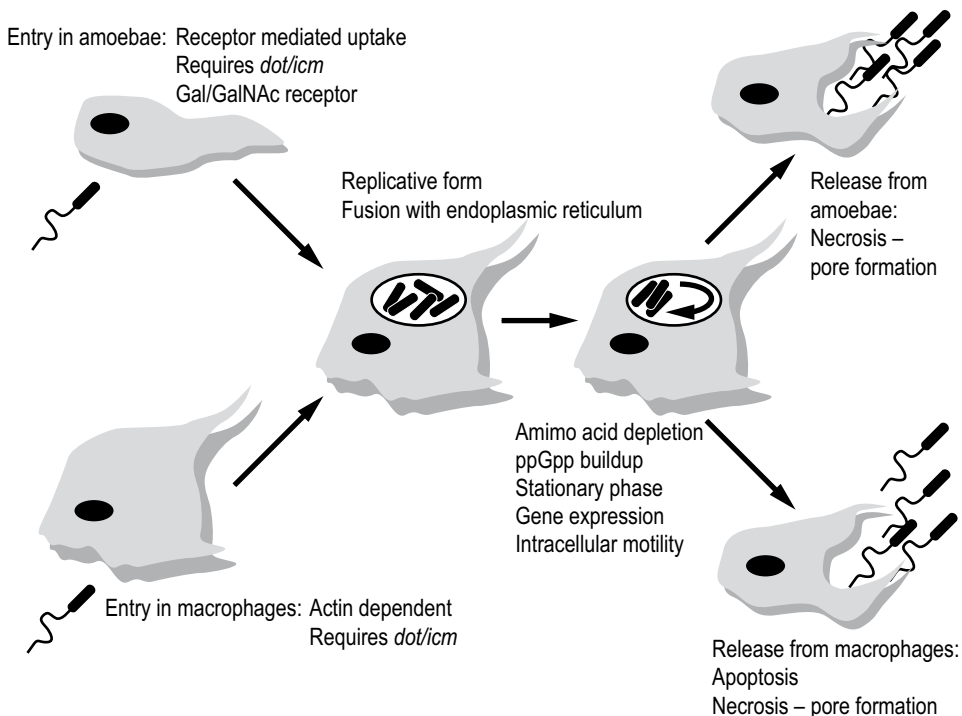
The virulence mechanisms of *L. pneumophila* are complex and not fully understood. Virulence is an important factor in the ability of *L. pneumophila* to infect and subsequently multiply within amoebae (Fields et al., 1986; Moffat & Tompkins, 1992). However, some strains with low virulence can multiply within certain host cells (Tully, Williams & Fitzgeorge, 1992). Studies contrasting the role that different virulence factors play in host populations may help to show how the bacteria develop an ability to infect humans, without the need for a protozoan host.

The interaction of virulent legionellae with phagocytic cells can be divided into several steps:

- binding of microorganisms to receptors on the surface of eukaryotic cells
- penetration of microorganisms into phagocytes
- escape from bactericidal attack
- formation of a replicative vacuole (a compartment within the cell where bacterial replication occurs)
- intracellular multiplication and killing of the host cell.

Legionellae have a similar life-cycle within protozoa and human macrophages; however, there are differences in the mechanisms used to enter and exit from the respective host cell types. These differences are summarized in Figure 1.1. Not all of the species of *Legionella* that have been studied are able to infect macrophages. However, *L. pneumophila* that possess the relevant virulence factors can infect and replicate within various protozoa found in soil and in water; and by replicating in this way they may become more virulent (Cianciotto, 2001).

Figure 1.1 Life-cycle of *Legionella pneumophila* in protozoa and human macrophages



Source: Fields, Benson & Besser (2002) (Reproduced with permission of authors)

Once *Legionella* enters the lung of an infected person (whether by aerosol or aspiration), both virulent and non-virulent strains are phagocytosed by alveolar macrophages and remain intact inside the phagocytes. However, only virulent strains can multiply inside the phagocytes and inhibit the fusion of phagosomes with lysosomes (Horwitz, 1993). This leads to death of the macrophage and the release of large numbers of bacteria from the cell. The bacteria can then infect other macrophages, thereby amplifying bacterial concentrations within the lungs. The pathogenesis of *L. pneumophila* has been made clearer by the identification of genes that allow the organism to bypass the endocytic pathways of both protozoan and human cells, although not all species investigated have this ability. Ogawa et al. (2001) studied six species of *Legionella* in Vero cells (a cell line developed from African green monkey nephrocytes). All species differed in morphology, implying that *Legionella* species may differ in their mode of intracellular multiplication.

During phagocytosis, legionellae initiate a complex cascade of activities, including:

- inhibition of the oxidative burst
- reduction in phagosome acidification
- blocking of phagosome maturation
- changes in organelle trafficking.

Legionellae thus prevent bactericidal activity of the phagocyte, and transform the phagosome into a niche for their replication (Stout & Yu, 1997; Sturgill-Koszycki & Swanson, 2000; Fields, Benson & Besser, 2002). The organisms can leave the host cell after temporal pore-formation-mediated lysis (Molmeret & Abu Kwaik, 2002) or can remain within an encysted amoeba (Rowbotham, 1986).

Two growth phases were described for one strain of intracellular *L. pneumophila*: the replicative non-motile form and the non-multiplicative motile form (Fields, Benson & Besser, 2002). Intracellular changes, such as host cell amino acid depletion and the subsequent accumulation of guanosine 3', 5'-bispyrophosphate (ppGpp) (Hammer & Swanson, 1999) resulted in the expression of stationary-phase proteins in one strain of *L. pneumophila* (although these findings may not apply to all strains), as shown in Figure 1.1. The proteins produced facilitate the infection of new host cells, affecting factors such as sodium sensitivity, cytotoxicity, osmotic resistance, motility and evasion of phagosome-lysosome fusion (Swanson & Hammer, 2000). The ability to infect host cells is also influenced by the expression of flagellin (Bosshardt, Benson & Fields, 1997), although the flagellar protein itself is not a virulence factor (Fields, Benson & Besser, 2002).

1.5.2 Surface structures involved in pathogenicity

Surface structures play an important role in the pathogenicity of *Legionella* (Cianciotto, 2001; Heuner et al., 2002). Adherence followed by entry of the bacterium into the host cell is the crucial step in the infection cycle. Together with the flagellum and the pili, certain bacterial surface proteins are involved in the adherence and entry of *Legionella* into alveolar macrophages and protozoa. These proteins include:

- the major outer membrane protein (MOMP)
- the heat shock protein (Hsp60)
- the major infectivity potentiator protein.

MOMP binds the complement component C3, and mediates the uptake of *L. pneumophila* via macrophage receptors for the complement components CR1 and CR3 (Heuner et al., 2002). Phagocytosis of *L. pneumophila* also occurs by a complement-independent mechanism (Weissgerber et al., 2003).

1.5.3 Virulence factors

Individual biological and immunological factors mediating virulence have not been explicitly defined (Stout & Yu, 1997; Yu, 2000). However, analysis of the infection process in protozoa and human host cells has identified certain general factors that may affect virulence, such as:

- expression of multiple proteins during infection of macrophages (Abu, Eisenstein & Engleberg, 1993)
- expression of certain proteases (Rechnitzer & Kharazmi, 1992; the proteases are thought to be important in the pathogenicity of *L. pneumophila*, but it is not clear whether they contribute to virulence)
- plasmids contained in *L. pneumophila*, which may affect intracellular survival (Bollin et al., 1985a; Chien et al., 2004).

One product of *Legionella* clearly associated with virulence is the 24-kDa macrophage infectivity potentiator (Mip) protein, coded for by the *mip* gene (Fields, 1996). The Mip protein is thought to be conserved throughout the genus (Cianciotto et al., 1989, 1990; Ratcliff et al., 1998); it is required for efficient infection of both mammalian phagocytic cells and protozoa (Cianciotto & Fields, 1992), but its mechanism of action is unknown.

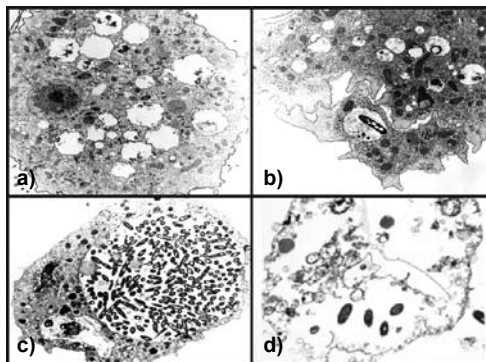
The type IV secretion system, a bacterial conjugation system used for transporting and injecting DNA or toxins into target cells, has a crucial role in the spread of pathogenicity. Within the loci encoding the type IV secretion systems (*dot/icm*) are 24 genes essential for infection of the host cell, and involved in assembling and activating conjugal transfer of plasmid DNA. *L. pneumophila* uses these operons to deliver virulence factors and a protein that diverts the

phagosome from its endocytic pathway (Fields, Benson & Besser, 2002). Genes such as *pilE* (coding for the pilin protein) and *pilD* (coding for prepilin peptidase) are important for unrestricted intracellular growth. Other loci involved in intracellular multiplication are *mak* (macrophage killing), *mil* (macrophage-specific infectivity loci), and *pmi* (protozoan and macrophage infectivity). Defects in any of these loci obstruct or interrupt intracellular growth (Sadosky, Wiater & Shuman, 1993; Gao, 1997; Gao, Harb & Kwaik, 1998; Fields, Benson & Besser, 2002).

Tissue-destructive protease is another important factor in the ability of *Legionella* to cause infection (Baskerville et al., 1986). Other factors that may increase virulence include several cytotoxins, heat shock proteins and compounds associated with iron uptake. The stationary phase response and the iron acquisition functions of *L. pneumophila* also play key roles in pathogenesis, as do a number of other loci, including the *pts* and *enh* genes (Cianciotto, 2001).

Virulence factors affect the ability of legionellae to grow within protozoa, as seen from studies showing the effect of incubating a virulent *L. pneumophila* strain and the corresponding avirulent strain with an *Acanthamoeba polyphaga* from a source implicated in an outbreak of Legionnaires' disease (Surman, Morton & Keevil, 1999; Surman et al., 2002). Figure 1.2 shows the organism after overnight incubation at 37 °C.

Figure 1.2 *Acanthamoeba polyphaga* isolated from a source implicated in an outbreak of Legionnaires' disease



Photograph courtesy of Dr S Surman-Lee

- a) No legionellae (control).
- b) An avirulent *L. pneumophila* strain when viewed by transmission electron microscopy (TEM). Some of the amoebae contain vacuoles with *L. pneumophila* inside; others contain degenerate material, including what appears to be the remains of *Legionella*. The amoebae are motile, with no signs of infection, and none has burst. These amoebae, apart from the presence of *Legionella* in the vacuoles, do not differ from the control.
- c) An avirulent *L. pneumophila* strain incubated with the corresponding virulent *L. pneumophila* strain. The legionellae have infected the *Acanthamoeba* and replicated within it, with many intracellular *L. pneumophila*.
- d) Damage caused to the amoeba by the cytotoxic activity of *Legionella*, which caused death of the amoeba.

1.5.4 Host defence

The host defence against *Legionella* relies principally on cell-mediated immune mechanisms. At least two proteins produced by *L. pneumophila* can induce protective cell-mediated immunity without being virulence factors — the major secretory protein (MSP, 39 kDa) and the major outer membrane protein (ompS, 28 kDa) (Blander & Horwitz, 1991). Circulating antibodies are produced during *L. pneumophila* infections in humans, but do not seem to be protective.

1.5.5 Transmission

An infected source (e.g. a fountain) can disseminate sprays or droplets of water containing legionellae, commonly referred to as aerosols. When this occurs, most or all of the water in the droplet evaporates quickly, leaving airborne particulate matter that is small enough to be inhaled. Particles of less than 5 µm in diameter can be deeply inhaled, and enter the respiratory airways to cause legionellosis (Fitzgeorge et al., 1983).

Legionella infections have frequently been associated with sources at distances of up to 3.2 kilometres (Addiss et al., 1989); recent evidence suggests that infection may be possible at even longer distances (Tran Minh et al., 2004). There is evidence that virulence is an important factor in the survival of *Legionella* in aerosols, with the most virulent strains surviving longer than their less virulent counterparts (Dennis & Lee, 1988).

There is no evidence of person-to-person transmission of either Legionnaires' disease or Pontiac fever (WHO, 2004).

Chapter 2 Ecology and environmental sources of *Legionella*

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A good understanding of the factors that affect *Legionella* survival and growth in the natural environment is important in controlling the bacteria in artificial water systems. It allows the areas most at risk from *Legionella* colonization in such systems to be identified, thereby indicating the points at which control measures will be most effective; it also allows the control measures that will be most effective to be identified (see Chapter 3).

This chapter discusses the relationship of *Legionella* with its natural environment, and provides information on:

- natural sources of *Legionella* (Section 2.1)
- factors affecting *Legionella* growth—water temperature and presence of other microorganisms (Section 2.2)
- how the formation of biofilms protects *Legionella* and supplies nutrients (Section 2.3)
- sources of *Legionella* infection — aerosols, other water sources and soil (Section 2.4).

2.1 Natural sources of *Legionella*

Understanding the ecology of *Legionella* (i.e. the way it interacts with its natural environment and with other species) helps in understanding the factors that encourage the survival and growth of legionellae in artificial water systems.

Legionellae are ubiquitous in natural and artificial water environments worldwide, and survive in a range of environmental conditions (Fliermans et al., 1981). The bacteria are acid-tolerant (they can withstand exposure to pH 2.0 for short periods) and they have been isolated from environmental sources ranging from a pH of 2.7 to 8.3 (Anand et al., 1983; Sheehan, Henson & Ferris, 2005). Legionellae have been found in sources as diverse as water on plants in rainforests, groundwaters (Riffard et al., 2001; Brooks et al., 2004) and seawater (Ortiz-Roque & Hazen, 1987). The bacterium also survives in artificial sources of salt water (Heller et al., 1998). In certain natural aquatic environments (e.g. in groundwater that is contaminated by soils or subsoils and has a temperature below 20 °C), legionellae may be present in concentrations

too low to be detected using culture methods. Such water can potentially introduce legionellae into storage tanks and systems within the built environment, where the physical and chemical conditions encourage their growth.

2.2 Factors affecting growth of *Legionella*

This section discusses the effect of temperature, other microorganisms and virulence factors on the growth of *Legionella*.

2.2.1 Influence of temperature

Legionellae have been isolated from hot-water systems up to 66 °C; however, at temperatures above 70 °C they are destroyed almost instantly (Dennis, Green & Jones, 1984; Dennis, 1988b). Kusnetsov et al. (1996) found that growth of all strains tested decreased at temperatures above 44–45 °C, with the growth-limiting temperature being between 48.4 °C and 50.0 °C. The *Legionella* strains studied produced carbon dioxide up to 51.6 °C, suggesting that some respiratory enzymes survive at this temperature. Complex water systems, such as warm-water plumbing systems, air-conditioners and hot tubs (also known as spa pools), are increasingly using water in the temperature range that encourages *Legionella* growth. In addition, these water systems can potentially produce aerosols, increasing the spread of the bacteria.

Strains of *L. pneumophila* have been shown to have a decimal reduction time (D)⁴ of 80–124 minutes at 50 °C, and of 2 minutes at 60 °C (Dennis, Green & Jones, 1984; Schulze-Robbecke, Rodder & Exner, 1987). Isolates can be collected easily from many different environmental aquatic sources with temperatures between 30 °C and 70 °C (Fliermans, 1984). For example, legionellae have been isolated from frozen rivers, thermal ponds and springs, and aquatic sources in the vicinity of a volcano (Tison & Seidler, 1983). Yee & Wadowsky (1982) showed that naturally occurring *L. pneumophila* survived and multiplied in water at temperatures between 25 °C and 45 °C, with an optimal temperature range of 32–42 °C. The study also found that legionellae were most commonly isolated at temperatures between 35 °C and 45 °C, with the greatest increase in viable counts occurring between 37 °C and 42 °C (Wadowsky & Yee, 1983; Schulze-Robbecke, Rodder & Exner, 1987). As the temperature falls below 37 °C, the bacteria's reproductive rate decreases and there is little or no increase in numbers of bacteria below 20 °C.

Therefore, to prevent *Legionella* infection, the recommended temperature for storage and distribution of cold water is below 25 °C, and ideally below 20 °C. Recent laboratory studies of mutant *Legionella* strains show that the bacteria may grow below 20 °C under certain conditions (Soderberg, Rossier & Cianciotto, 2004). *Legionella* will survive for long periods at low temperatures and then proliferate when the temperature increases, if other conditions allow.

⁴ The “decimal reduction time” is a unit of microbial heat resistance, defined as the time required to kill 90% of a population of microorganisms at a constant temperature and under specified conditions.

L. pneumophila is thermotolerant and able to withstand temperatures of 50 °C for several hours.

The identification of *Legionella* spp. in hot-water tanks or in thermally polluted rivers emphasizes that water temperature is a crucial factor in the colonization of water distribution systems (Yu, 2000), the proliferation of legionellae in the environment, and therefore the risk of *Legionella* infection. Maintaining the temperature of hot and cold-water systems within buildings to prevent or minimize the growth of legionellae is an important control measure to prevent the risk of *Legionella* infection.

2.2.2 Effect of other microorganisms

Requirement for nutrients

Water alone is insufficient to allow *L. pneumophila* to proliferate; for example, in studies using sterile distilled water and sterile tap water, *L. pneumophila* survived in the long term but did not multiply (Skaliy & McEachern, 1979; Fields et al., 1984). Other microorganisms allow *Legionella* to amplify; for example, naturally occurring *L. pneumophila* were able to survive and multiply in non-sterile tap water (Yee & Wadowsky, 1982). In continuous-culture model systems seeded with a mixed microflora from a potable water system, *L. pneumophila* grew when fed solely with filtered, sterilized drinking water for prolonged periods (Lee & West, 1991; Rogers et al., 1994). These results suggest that growth of *Legionella* requires nutrients already available in the tap water. The nutrients may be supplied, directly or indirectly, by other species of bacteria or other associated microorganisms in the form of dissolved organic constituents, through the excess production of organic nutrients or through decay of the microorganisms (Tesh & Miller, 1981; Yee & Wadowsky, 1982; Stout, Yu & Best, 1985):

These results are consistent with studies showing that amino acids are the main nutrient requirement for *L. pneumophila* growth (Pine et al., 1979; Warren & Miller, 1979; Wadowsky & Yee, 1985).

The association of *L. pneumophila* with many different microorganisms from aquatic sources has been demonstrated; the microorganisms include protozoa, *Fischerella* spp. and other bacteria (Fliermans et al., 1981; Tesh & Miller, 1981; Bohach & Snyder, 1983; Wadowsky & Yee, 1983; Wadowsky & Yee, 1985; Rowbotham, 1986; Grimes, 1991).

Protozoa

Drozanski (1963) described bacterial parasites of amoebae that had been isolated from soil but failed to grow on laboratory media. It is possible that these bacterial parasites were *Legionella* spp. Rowbotham (1980) was the first to report the relationship between amoebae and *L. pneumophila*; it has subsequently been confirmed that legionellae are facultative intracellular parasites. (Facultative organisms are those that are able to grow in altered environmental conditions, for example, in the presence or absence of a specific environmental factor, such as oxygen.)

Legionellae can multiply in 14 species of protozoa, including:

- *Acanthamoeba*, *Naegleria* and *Hartmanella* spp.
- the ciliates *Tetrahymena pyriformis*, *Tetrahymena vorax* (Rowbotham, 1980; Tyndall & Domingue, 1982; Fields et al., 1984; Rowbotham, 1986; Wadowsky et al., 1991)
- one species of slime mould (Rowbotham, 1980; Fields et al., 1993; Fields, Benson & Besser, 2002).

Protozoa are an important vector for the survival and growth of *Legionella* within natural and artificial environments, and have been detected in environments implicated as sources of legionellosis. However, not all amoebae are acceptable hosts, indicating that a degree of host specificity is involved. In the natural environment, *L. pneumophila* proliferates in protozoa within intracellular phagosomes, possibly producing proteases with cytotoxic activity, and thus causing localized tissue destruction (Quinn, Keen & Tompkins, 1989).

Once it has been ingested by an amoeba, the survival of *L. pneumophila* depends on the temperature of the water. At 22 °C, the bacteria are digested by the amoeba (Nagington & Smith, 1980), whereas at 35 °C they can proliferate inside the amoeba (Rowbotham, 1980). Temperature also affects the expression of flagella, with a larger number of flagellated bacteria present at 30 °C than at 37 °C (Ott et al., 1991). Flagella have an important role in the pathogenicity of many organisms, including *Salmonella* and *Pseudomonas aeruginosa*. Heuner & Steinert (2003) found that nonflagellated legionellae were less capable of infecting protozoa and macrophages than wild-type flagellated strains.

Protozoa help to protect *Legionella* from the effects of biocides (Barker et al., 1992) and thermal disinfection (Storey, Ashbolt & Stenstrom, 2004a). Legionellae can survive in encysted amoebal cells (Skinner et al., 1983; Harf & Monteil, 1988) and it has been postulated that this can be a mechanism by which *L. pneumophila* is able to survive adverse environmental conditions and survive within airborne aerosols (Berendt, 1980; Hambleton et al., 1983; Tully, 1991).

Phagocytic cells

The virulence of *Legionella* is linked to its capacity to proliferate in humans, where it infects phagocytic cells opportunistically (i.e. taking advantage of certain conditions to cause disease). However, these studies preceded the recognition of serological cross-reaction between *L. pneumophila* and *Campylobacter* spp. Infection of susceptible animals such as guinea pigs, rats, mice and hamsters has shown that the pattern of growth in macrophages is similar to that in protozoa. The bacterium has been isolated from the lungs of calves, and serological conversion has been observed in many animals, including horses, antelope and sheep (Boldur et al., 1987). Therefore, infection is not solely caused by the virulence of *L. pneumophila*, but can also depend on the susceptibility of the host. Attempts to infect birds (quails and pigeons) with *L. pneumophila* were unsuccessful (Arata et al., 1992).

2.2.3 Environmental factors and virulence

The virulence mechanisms of *Legionella* are discussed in Chapter 1. Virulence is influenced by environmental factors such as temperature, nutrients and sodium concentrations (Edelstein, Beer & DeBoynton, 1987; Byrne & Swanson, 1998). At the same time, *Legionella*'s virulence factors affect the ability of the bacteria to survive adverse environmental influences, such as temperature extremes (Mauchline et al., 1994), ultraviolet (UV) light, low humidity and biocide treatments (Rowbotham, 1980; Anand et al., 1983; Rowbotham, 1984; Barbaree et al., 1986). Chapter 1 (Section 1.5) provides more information on environmental factors and virulence.

2.3 Biofilms

This section discusses the composition and formation of biofilms, their effect on bacterial growth, and risk factors for the development of biofilms.

2.3.1 Biofilm composition

In 1901, Whipple noted how adherence to surfaces increased the bacterial activity of waterborne microorganisms. Since then, many studies have recognized the importance of surfaces in concentrating microorganism activity. Surface-associated microbial activity and colonization, or “biofilm formation”, occurs worldwide in natural and artificial environments, and on a range of different surfaces. Microorganisms, including *L. pneumophila*, form biofilms as a mechanism to withstand adverse conditions, such as limited nutrients or temperature extremes. Surface adherence usually occurs by means of an extracellular polysaccharide substance secreted by the cells. This substance (the glycocalyx, or slime) is a hydrated polyanionic polysaccharide matrix produced by polymerases affixed to the lipopolysaccharide component of the cell wall (Morton et al., 1998).

At any stage in a biofilm's development, portions of the film can be sloughed off by shear stresses from the movement of water (see Figure 2.1) (Trulear & Characklis, 1982; Taylor Eighmy & Bishop, 1985). This activity may resuspend the biofilm's microorganisms within the system's water (Rowbotham, 1980), allowing them to colonize other parts of the system if conditions are appropriate.

Microbial biofilms are extremely complex heterogeneous microbial ecosystems and may consist of bacteria, algae and grazing protozoa. The latter may display morphological features not usually associated with microorganisms when grown in pure culture (Cloete et al., 1989).

2.3.2 Biofilm formation

During biofilm formation, the surface to which the film will attach is first conditioned by nonspecific binding; this process is followed by colonization of pioneering microorganisms, which multiply to form microcolonies or stacks. The microcolonies are protected by a glycocalyx layer, but portions can be sheared off and recolonize other parts of the system, as described

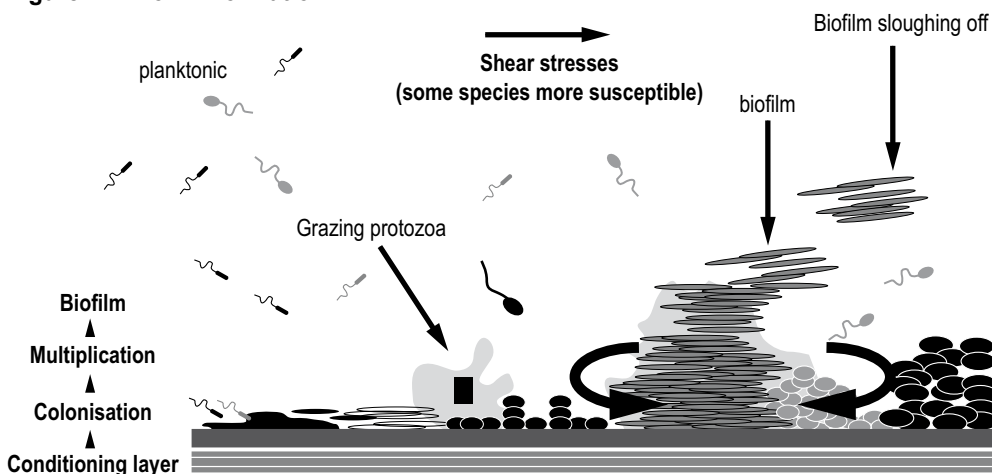
above. Fluid flow around the microcolonies (represented by curved arrows in Figure 2.1) carries nutrients, and the surface is grazed by protozoa (if present), which releases nutrients and clears surfaces, thus aiding growth.

Biofilms, which may include legionellae and protozoa, can form on the surfaces of poorly managed buildings or cooling towers (see Figure 2.1). The biofilm facilitates nutrient and gaseous exchange, and protects microorganisms not only from biocides but also from periodic increases in temperature and attempts at physical removal, especially in areas where surfaces are scaled or corroded. Biofilms can form at interfaces, particularly at those between water and solid surfaces, but have also been found on oil–water interfaces (e.g. in metal-working fluids). Biofilms are more likely to form where there are areas of low water flow and where water is allowed to stagnate.

Studies aimed at characterizing bacterial interaction within biofilm ecosystems have evaluated the effects of parameters such as temperature and surface materials on the growth of *L. pneumophila*, and have investigated the effect of biocides on planktonic and sessile legionellae (those attached to the surface material) (Green & Pirrie, 1993; Walker et al., 1993, 1999; Rogers et al., 1994; Moorer, 1996; Atlas, 1999; Surman, Morton & Keevil, 1999; Murga et al., 2001; Keevil, 2003). Most studies of *Legionella* and biofilms use naturally occurring microbial communities, and therefore give a true picture of such communities (Colbourne et al., 1984; Colbourne & Dennis, 1985; Verissimo et al., 1990; Storey, Ashbolt & Stenstrom, 2004b). However, some of the organisms present in biofilms have yet to be identified, and their contribution to the survival and multiplication of legionellae remains unknown.

Within a biofilm, microorganisms are embedded in an extracellular matrix that provides structure, stability, nutrients and protection from possible toxic effects of the substrate upon which the biofilm grows (e.g. copper pipes in water distribution systems). Gradients of nutrients, pH and oxygen within the matrix support the varying needs of different microorganisms in the heterogeneous population (Wimpenny, Manz & Szewzyk, 2000; Allison, 2003). Legionellae grown in biofilms are more resistant than the same bacterial species in the water phase of the system (Barker et al., 1992; Cargill et al., 1992; Surman, Morton & Keevil, 1993; Santegoeds, Schramm & de Beer, 1998).

Figure 2.1 Biofilm formation



Source: Kindly supplied by Susanne Surman-Lee

2.3.3 Effect of biofilms on bacteria growth

Bacteria attached to surfaces and particulate matter within a system are more resistant to biocide treatments (Ridgway & Olson, 1982; Kuchta et al., 1985; King et al., 1988), making biocides less effective and allowing the proliferation of potential pathogens (LeChevallier et al., 1988; Wright et al., 1991).

The presence of biofilms is therefore an important factor for *Legionella* survival and growth in water systems (Kramer & Ford, 1994; Rogers et al., 1994; Williams, Molinari & Andrews, 1996; Martinelli et al., 2000; Goossens, 2001). Small numbers of legionellae are found in sources such as distributed drinking-water supplies, which then feed into water systems within buildings and cooling towers. This provides a logical explanation for the presence and subsequent growth of legionellae in these artificial aquatic environments (ASHRAE, 2000; WHO, 2004).

The availability of complex nutrients in biofilms has led some researchers to propose that biofilms support the survival and multiplication of legionellae outside a host cell. Growth within a biofilm composed of naturally occurring waterborne microorganisms, in the absence of protozoa, has been shown in a model system study. Cycloheximide — which inhibits protein synthesis in all eukaryotic cells, and affects initiation, elongation and termination, (Oleinick, 1977) — was added in high doses to the system. Growth increased in the absence of protozoa, with both the heterotrophic count (the number of all microorganisms) and the *Legionella* count increasing (Surman, Morton & Keevil, 1999; Surman et al., 2002). Rogers & Keevil (1992) used immunogold labelling of *Legionella* to show the existence of microcolonies of legionellae within biofilms. Another study demonstrated that multiplication of *Legionella* in a biofilm model was due solely to intracellular multiplication in amoebae (Murga et al., 2001).

2.3.4 Risk factors for biofilm growth

Biofilm prevention is an important control measure against proliferation of *Legionella*. Preventing the growth of biofilms is important because, once established, they are difficult to remove from complex piping systems (see also Chapter 4).

Various factors increase the likelihood of biofilm formation, including:

- the presence of nutrients, both in the source water and in the materials of the system
- scale and corrosion
- warm water temperatures
- stagnation or low flow as occurs in the deadends of distribution system pipework and in storage tanks.

The presence of scale and corrosion in a system will increase the available surface area and allow the formation of microniches that are protected from circulating disinfectants. Scale and corrosion also increase the concentration of nutrients and growth factors, such as iron, in the water system. Uncontrolled biofilms can occlude pipework, resulting in areas of poor flow and stagnation with higher risk of *Legionella* growth. Furthermore, the presence of both biofilms and protozoa has a twofold protective effect for the bacteria in the system, because it increases the organic load and inactivates residual levels of disinfectant. In addition, biofilm and bacteria (including *Legionella* spp.) grown inside protozoa are more tolerant of chlorine and other antimicrobial agents at concentrations above those commonly used to disinfect water supplies and shown to be lethal under laboratory conditions (Barker et al., 1992).

The materials of the system also affect the growth of biofilms. Some plumbing materials support or enhance the proliferation of microorganisms, including *Legionella* spp. (Rogers et al., 1994). Natural substances, such as rubber gaskets, provide a nutrient-rich substrate and are preferentially colonized by microorganisms; some plastics leach nutrients into the system. Microorganisms will even grow on the surface of systems plumbed with copper, which has an inherent resistance to colonization, once the surface has been conditioned.

Most engineered aquatic systems — especially those that are complex (e.g. those in health-care facilities and hotels) — have areas containing biofilms, even when the system is well maintained. When control measures, such as the disinfection regime, are relaxed, microorganisms will quickly multiply to detectable levels.

Legionella contamination can originate from small areas of a water system that are not exposed to temperature fluctuations or circulating disinfectant. An example of this occurred in a large teaching hospital in the United Kingdom, where legionellae were intermittently detected at one sentinel outlet, despite the fact that there was a comprehensive control regime in place. The source was eventually tracked down to a 10-centimetre length of water-filled pipe where there was little or no flow (a “deadleg”). When this section of pipe was removed, subsequent sampling remained negative (John Lee, Health Protection Agency, UK, personal communication, June 2005).

2.4 Sources of *Legionella* infection

It is not possible to predict whether a source will cause infection based solely on the *Legionella* count. The likelihood that a source will cause an infection depends on the load of bacteria, the effectiveness of dissemination, the way in which it multiplies, and its ability to form aerosols.

2.4.1 Disease spread via aerosols and inhalation

The role of aerosols from contaminated potable water distribution systems in leading to legionellosis is well established. Other chapters of this publication (see Chapters 4–8) discuss the many aerosol-generating systems that have been linked with transmission, such as cooling towers, building water systems, respiratory therapy equipment and hot tubs.

Showers are often mistakenly thought to be the only source of aerosols linked to nosocomial legionellosis (Woo, Goetz & Yu, 1992); however, water outlets, humidifiers, respiratory devices and nebulizers that have been filled or cleaned with tap water can also spread *Legionella* and have been reported as a source of infection in several cases (Arnow et al., 1982; Moiraghi et al., 1987; Brady, 1989; Mastro et al., 1991; Woo, Goetz & Yu, 1992). Toilet flushing is also a potential source (Albrechtsen, 2002).

As discussed in Chapter 1, community-acquired cases of legionellosis can almost always be attributed to inhalation of aerosols from devices such as cooling towers, hot tubs, industrial equipment and indoor fountains (Heng et al., 1997; Den Boer et al., 2002; Greig et al., 2004). The largest outbreaks of disease to date have all been associated with transmission of aerosols from these types of equipment (Den Boer et al., 2002; Garcia-Fulgueiras et al., 2003; Greig et al., 2004). Cooling towers are a particular problem, with one report suggesting that cooling towers account for at least 28% of all sporadic cases of legionellosis (Bhopal, 1995).

Other systems implicated in the spread of legionellosis via aerosols include domestic plumbing systems (Singh, Stout & Yu, 2002; WHO, 2004; see Chapter 4); misting devices associated with food displays (Mahoney et al., 1992), natural thermal springs (Sommese et al., 1996; Alim, Hakgudener & Poyraz, 2002) and thermal spas (Brady, 1989; Martinelli et al., 2001; Vogianis et al., 2004).

As discussed in Chapter 1, nasogastric tubes have been included in several studies of nosocomial legionellosis, with microaspiration of contaminated water presumed to be the mode of entry (Marrie et al., 1991; Blatt et al., 1994; Stout & Yu, 1997). However, a recent study failed to detect colonization of the oesophageal tract by *Legionella* in this situation (Pedro-Botet et al., 2002). Patients suffering from nosocomial legionellosis are significantly more likely to have undergone endotracheal tube placement, or to have been intubated for significantly longer, than patients with other causes of pneumonia (Strebel et al., 1988; Kool et al., 1998; Winston, Seu & Busuttill, 1998).

2.4.2 Disease spread via soil

In a number of documented cases of legionellosis, no aquatic source was implicated. In these cases, likely sources of infection have been potting soils and soil conditioners. Most reports of soil-derived infection since the 1990s identify *L. longbeachae* as the infectious agent (Steele, Lanser & Sangster, 1990; Steele, Moore & Sangster, 1990; Koide et al., 1999). The mode of transmission of these infections remains unclear.

Anecdotal reports suggest possible links between building excavations and outbreaks of legionellosis (Miragliotta et al., 1992; Mermel et al., 1995). These outbreaks may be due to increased dispersion of dust during earthmoving operations, since dust entering cooling towers adds nutrients and surfaces for bacterial growth and may also interfere with biocide action. Alternatively, the outbreaks may be due to interference in the water supply, which allows contamination by bacteria, including legionellae.

Chapter 3 Approaches to risk management

Jamie Bartram, Richard Bentham, Emmanuel Briand, Phil Callan, Sebastian Crespi, John V Lee, Susanne Surman-Lee

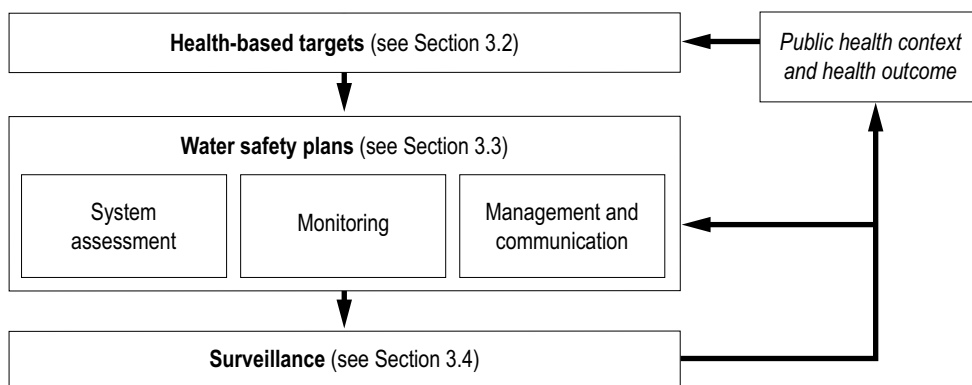
The World Health Organization (WHO) has developed a framework for safe drinking-water that can be applied to assessing and managing the risks posed by *Legionella*. Figure 3.1 illustrates this framework.

This chapter first considers the links between environmental exposure to *Legionella* and outbreaks of disease (Section 3.1), and then describes how the framework can be used to minimise the risk of *Legionella* colonizing a water system. The framework has the following components:

- *health-based targets* (Section 3.2) — these are targets normally set at national or state level by a competent authority, either in the health sector, or in consultation with the health sector
- *water safety plans (WSPs)* (Section 3.3) — these are system specific plans developed and implemented by the operator of the system (in the case of *Legionella*, such plans may be building specific, and may be developed and implemented by the building operator)
- *surveillance* (Section 3.4) — this is a system of independent checking, by a surveillance body or regulatory agency.

The information on health-based targets and surveillance is similar for all types of situation where *Legionella* may be found; however, a WSP is necessary for each particular situation. Therefore, Chapters 4 to 8 discuss the application of WSPs for *Legionella* to particular situations.

Figure 3.1 Framework for safe drinking-water



Source: WHO (2004)

3.1 Environmental exposure and disease

There is no established dose–response relationship for *Legionella* infections, and the concentration of legionellae necessary to cause an outbreak is unknown. Transmission may occur through inhalation, aspiration or directly from contaminated water (e.g. wound infections), from a wide variety of sources, as discussed in Chapter 1. Data on population dynamics indicate that legionellae are not distributed normally within the aquatic environment, and that even when high concentrations of the bacteria are detected, this may not be related to health risk (Kool et al., 1999; Bentham, 2000). Gathering information on population dynamics of *Legionella* is difficult, because there have been relatively few outbreaks in which the source has been investigated while it was still infectious, and in which no intervention has occurred before sampling.

Of the many reports of *Legionella* outbreaks caused by cooling towers, few provide details of the numbers of legionellae present in the water at the time the tower was infectious. Often, the tower was examined long after the infectious period, and the bacterial population may have changed dramatically in the interim, as shown by the example in Box 3.1.

Box 3.1 Hospital outbreak in which water sampling was ineffective

In 1985, an outbreak of legionellosis occurred at Stafford District General Hospital in the United Kingdom. The investigation team was able to isolate *L. pneumophila* from a piece of sealant in an air handling unit, but not from any water sample (O'Mahony et al., 1990). Presumably, at the time of the outbreak, the cooling water contained high levels of *L. pneumophila*. However, between the time of the outbreak and the arrival of the investigation team, the cooling water had been shot dosed (given a brief, high-level treatment) with biocide at least twice, and had been diluted by fresh make-up water. Numbers of legionellae would have been reduced considerably by both the biocide and the dilution with fresh water. Low numbers of *L. pneumophila* were isolated from a sample of the cooling water collected between the shot doses. The sealant from which the investigation team isolated *L. pneumophila* came from around a chiller battery within the air-conditioning ducting. The position of the sealant meant that it could have been contaminated by aerosols from the cooling tower, but would not have been affected by the biocide additions.

3.1.1 Cooling tower outbreaks

In cooling tower outbreaks in the 1980s, numbers of bacteria were often estimated by immunofluorescence rather than by culture. These results may have been unreliable, because the reagents used were polyclonal antibodies, which have questionable specificity; also, the technique detected both dead and live legionellae. However, isolation of legionellae by culture tends to underestimate the numbers of legionellae by at least an order of magnitude.

Table 3.1 shows the results of various cooling tower outbreaks in which the towers were sampled while probably still containing infectious legionellae.

Table 3.1 Cooling tower outbreaks

Facility, location, date	Organism, concentration (CFU/litre)	Comments	Reference
British Broadcasting Corporation (BBC), London, UK, 1998	Up to 10^6 CFU/l by culture, and up to 10^9 CFU/l by immunofluorescence	In both outbreaks, the cooling towers had severely damaged drift eliminators ^b that would have effectively increased the dispersal of the infectious aerosol from the towers. People were infected up to 500 m from the BBC cooling tower. In both cases, towers were sampled while still infectious.	Westminster Action Committee, (1988)
British Aerospace, Bolton, UK, 1998	10^5 CFU/l by culture, and 10^7 CFU/l by immunofluorescence		Mitchell et al. (1990)
Community-acquired, Wisconsin, USA	<i>L. pneumophila</i> serogroup 1 10^6 in cooling tower	Towers sampled before disinfection, probably while still infectious. Epidemiological evidence suggested that patients were infected up to one mile (1.6 km) from the tower	Addiss et al. (1989)
Retirement hotel, Los Angeles, USA, July 1988	<i>L. pneumophila</i> 9×10^6 in water from evaporative condenser	Air around the building sampled while tower was operating and still infected. Patients possibly infected by as little as 0.02 CFU/l in air.	Breiman et al. (1990)
Two outbreaks of legionellosis from a single tower in Wisconsin, USA, October 1986	$>1.6 \times 10^6$ CFU/l (mean of 2 outbreaks), compared to controls (86/99 controls had $<5 \times 10^5$ CFU/l and 68/99 controls had means of $<10^5$ CFU/l)	Legionellae counts in likely sources of the outbreaks (cooling towers and evaporative condensers) significantly higher than in controls and towers not associated with reports of disease.	Shelton, Fanders & Morris (1994)
Community outbreak, caused by hospital cooling towers in Delaware, USA, 1994	<i>L. pneumophila</i> serogroup 1 at 2.32– 9.15×10^6 CFU/l in main tower; 1.05– 2.34×10^6 CFU/l in small tower	Incriminated towers examined while still infectious. Risk of illness 20% less for each 0.1 mile (160 m) increase in distance from the hospital, up to one mile away. Transmission mainly within 0.25 miles (400 m) of the cooling towers. Infection associated with frequent and extended exposure to the source, suggesting cumulative exposure as a risk factor for illness, as well as proximity to the source.	Brown et al. (1999)
Hotel outbreak in Sydney, Australia April 1993	<i>L. pneumophila</i> 2.8×10^7 CFU/l and 3.4×10^6 CFU/l in the two towers implicated		Bell et al. (1996)

CFU = colony forming unit

Bhopal et al. (1991) studied sporadic cases of legionellosis (i.e. those not associated with known outbreaks or with travel) in relation to the distance between the person's home and cooling towers. The study found that risk of infection decreased with increasing distance. People living within 0.5 km of any tower were three times more likely to become infected than people living more than 1 km away.

Bentham & Broadbent (1993) reviewed the common features of some community outbreaks associated with cooling towers, and found that towers implicated in outbreaks were mainly those of less than 300 kilowatts. Outbreaks were most frequent in autumn, and often involved towers that had been operated after a period of shutdown. The study monitored the numbers of legionellae in systems that had been shut down, with samples taken before, 10 minutes after and 70 minutes after switching on the circulation. In some cases, switching on the system raised *Legionella* concentrations from below the detection limit (4000 CFU/litre) to between 5.0×10^4 and 9.5×10^5 CFU/litre within 10 minutes.

3.2 Health-based targets

Health-based targets are based on critical evaluation of health concerns; for example, for *Legionella* safety, an overall health-based target might be to have "no cases of legionellosis caused by artificial water systems". Health-based targets for *Legionella* safety are normally set nationally and applied locally. Targets should be set by a senior authority responsible for health, in consultation with relevant experts, including environmental microbiologists, engineers, system designers and installers, maintenance staff and contractors, and people responsible for ensuring the health and safety of systems.

Health-based targets usually focus on controlling the proliferation of legionellae and the production and release of aerosols, because of the difficulty of determining what represents an acceptable limit for *Legionella*. For example, the cooling tower outbreaks listed in Table 3.1, above, all occurred at levels of at least 10^5 CFU/litre (by culture), but it would be dangerous to assume that it is safe to set an acceptable limit just below that level, because numbers could increase rapidly if a system is not adequately controlled. Also, environmental conditions may modulate the virulence of individual strains (Byrne & Swanson, 1998), and routine culture does not differentiate between virulent and avirulent strains. Thus, the public health significance of a culture result from a water sample cannot be determined, because the result is not necessarily related to virulence, exposure concentration, survival of the organism in an aerosol or the infectious dose of the organism.

A further issue is that culture methods are biased towards the species currently recognised to be associated with disease, particularly *L. pneumophila*, and may not detect all legionellae present in the environment. In addition, people vary in their susceptibility to infection, making it difficult to assess generic risk for the population at large. Thus, health risk assessments must be made without reference to the relationship between dose and response, and with only limited reference to test results.

Even when a source reaches a state at which it is infective, the proportion of people who acquire Legionnaires' disease is small (usually less than 5% of those exposed). Conversely, in outbreaks of Pontiac fever, a high percentage (about 95%) of those who are exposed become affected. A preliminary risk assessment by Ambroise & Hartemann (in press) compared exposure linked to aerosols produced by cooling towers and by showering. The study considered expected numbers of cases of infection, clinical sickness and death, for similar concentrations of *L. pneumophila* serotype 1 in the air (ranging from 0.02 to 200 CFU/m³). The authors found that exposure through cooling towers led to more cases (by a factor of 100–130) than exposure during showering.

3.3 Water safety plans

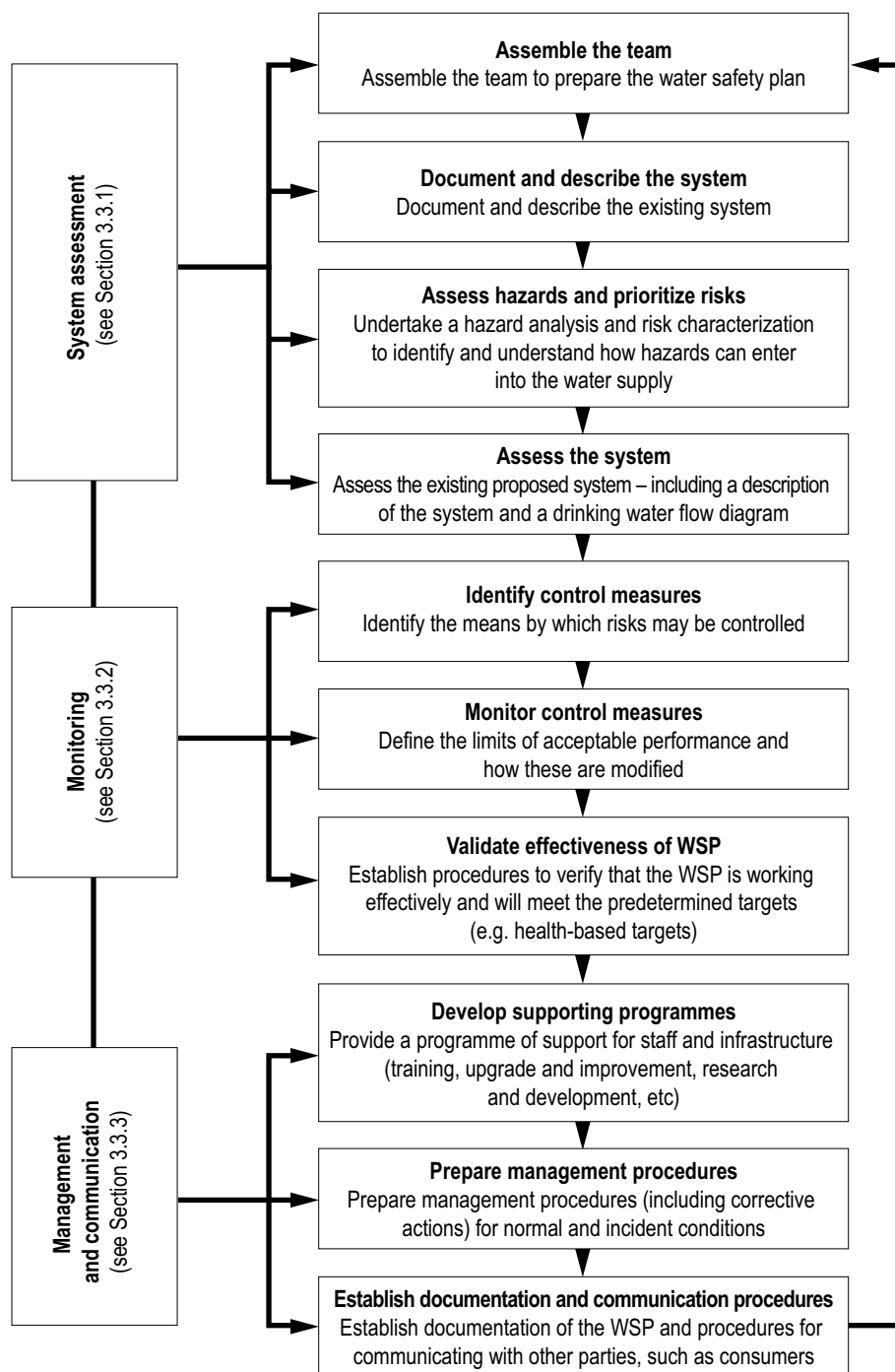
Developing a WSP is the preferred approach to managing specific health risks of exposure to *Legionella* from water systems (WHO, 2004; Davison et al., 2005). In some jurisdictions, other terms are used; for example, the term “risk management plan” is used by the Department of Human Services, Victoria, Australia. Such plans are similar to a WSP, but are less clearly defined. For the purposes of this document, the term WSP is used.

Authorities responsible for water system safety or building safety should develop system-specific WSPs. Major benefits of developing and implementing such a plan are the systematic and detailed assessment and prioritization of hazards (biological, chemical or physical agents, or water conditions, with the potential to cause adverse health effects), and the operational monitoring of barriers and control measures.

The steps involved in developing a WSP are shown in Figure 3.2. A plan consists of the following key components:

- *system assessment* (Section 3.3.1) — determination of whether the water quality at the point(s) of potential exposure or use meets the health-based target, based on a risk assessment for the population likely to be exposed
- *monitoring* (Section 3.3.2) — identification and monitoring of control measures used to ensure water safety (e.g. biocide levels, temperature, pH)
- *management and communication* (Section 3.3.3) — to document the system assessment and monitoring, and describe actions to be taken during normal operation and after incidents, including documentation and communication (e.g. a plan for remedial actions after adverse monitoring results, such as low residual biocide levels, and listing those to be informed of an event).

Figure 3.2 Overview of the key steps in developing a water safety plan



Source: adapted from WHO (2004)

The WSP should be prepared in conjunction with, and made available to, all concerned parties (e.g. health authorities, water suppliers, building managers and water treatment providers). The plan should be reviewed on a regular basis to reflect changes and ongoing improvements in the system, the available evidence base and the surrounding environment (WHO, 2004). Finally, the plan should be amended if control is not maintained.

3.3.1 System assessment

Assessment of the system supports subsequent steps in the plan, allowing effective strategies for controlling hazards to be developed and implemented. The steps involved — shown in Figure 3.2, and discussed below — are to:

- assemble a team
- document and describe the system
- assess hazards and prioritize risks
- assess the system.

Assemble a team

As shown in Figure 3.2, a preliminary stage in developing a WSP is to form a team of experts with a thorough understanding of the particular water system. A thorough knowledge and understanding of the system's operation is also critical. This should incorporate knowledge of design strengths and weaknesses and operating characteristics, so that informed decisions can be made about system maintenance and monitoring.

The training and experience of assessors are important factors in the quality of system assessments. Ideally, assessors should be independent of those who supply water treatment services, to avoid conflicts of interest. Assessors must be aware of the ecological factors that encourage *Legionella* growth within a system (see Chapter 2), and have some understanding of the design and engineering of the system, and of any modifications or alterations to the system, particularly if the system is large and complex. Assessment of complex systems will generally require a broad knowledge base and is best conducted by a multidisciplinary team that can address all aspects of system operation and management, including microbiological aspects. However, a team-based approach might not be feasible in some cases, for example where resources are limited. Therefore, a system assessment should establish the type and level of control that can realistically be imposed.

Document and describe the system

Large water systems, such as building water systems, are those most commonly associated with widespread human exposure to *Legionella*. Identifying the layout and design of such water systems is therefore an important step in controlling colonization, although the task can be time consuming and difficult. Due to the high level of technical capability required,

it may be useful to subcontract this task to a specialist contractor. Independent assessment of larger water systems will also help to identify design faults and areas that need servicing.

Routine servicing and replacement of components of the system should comply with manufacturers' specifications or existing technical references, where available, and should be carried out by properly qualified people. System layout and design specifications should be used to determine the servicing and replacement requirements for the entire system.

Assess hazards and prioritize risks

Each system should be assessed individually, taking into account the proximity and susceptibility of the population, and the mode of transmission from the water source. The potential risks associated with the system should also be evaluated. This step involves understanding the characteristics of the water system, the hazards that may arise and how they may create risks, and the processes and practices that affect water quality.

Assess the system

This step involves assessing the existing system, including describing the system and preparing a flow diagram. The aim of preparing a flow diagram is to increase the accuracy of the water system evaluation and provide a conceptual understanding of the water supply process. The diagram — a systematic representation of the sequence of steps or operations used in the production or manufacture of a particular water item — can be used to show:

- pathways by which legionellae can be transferred to consumers
- points where controls are in place and where improvements might need to be made
- links, water flow direction and responsibilities in the water supply process; for example, where the utility's responsibility ends (e.g. at the consumer's meter) and the consumer's begins (e.g. after receipt of water).

To avoid duplication, the diagram should cross-reference any supporting documentation that covers finer details. Such documentation might include geographical information system (GIS) layers and plumbing schematics, which could be used to identify stagnation points; for example, maps showing key account holders, such as hospitals, schools and nursing homes.

3.3.2 Monitoring

The steps involved in monitoring— shown in Figure 3.2, and discussed below — are to:

- identify control measures
- monitor control measures
- validate effectiveness of WSP.

Identify control measures

Control measures are activities or processes applied to a system to prevent a hazard occurring. Such measures are applied at control points, which are steps at which control can be applied to prevent or eliminate a water safety hazard or reduce it to an acceptable level. Some plans contain key control points; that is, points at which control is essential to prevent or eliminate a hazard.

Control measures for microorganisms in industrial systems have been described by Eggins & Oxley (1982), and include:

- excluding the microorganism
- manipulating the environment to prevent colonization by, and limit growth of, the microorganism (e.g. by controlling nutrient levels, controlling temperature, and preventing low flow and stagnation)
- manipulating the environment to limit growth of the microorganism
- using a disinfectant (e.g. a biocide).

The remainder of this subsection discusses how these strategies can be used to control *Legionella*. Any WSP would be based on a combination of control methods, rather than relying on any single method.

Exclusion of microorganisms

In most systems, it is not practical to exclude legionellae or to prevent their periodic reintroduction, because low numbers of *Legionella* may enter a building through piped distribution systems or storage systems. Therefore, emphasis must be upon design and control.

Control of nutrient levels

Limiting the amount and type of nutrients (particularly organic nutrients) that are available to the bacteria in the water system is an important control measure. Nutrient levels can be controlled by:

- selecting materials that will not serve as substrates or provide nutrients for biofilm development
- ensuring that chemical additives used to control scaling, corrosion and microorganisms are applied at appropriate and effective concentrations (Crespi & Ferra, 1997), and are chemically compatible (i.e. nonreactive) with one another and with the system
- considering the properties of materials used in the water system (e.g. insulating properties, potential for corrosion, interaction with chemical disinfection processes)
- ensuring that system design is appropriate and will prevent the accumulation of biofilms, sediments and deposits (e.g. the design should eliminate deadends and stagnation, and allow access to all parts of the water system for maintenance and cleaning).

Prevention of low flow and stagnation

Preventing low flow rates and stagnation of water is an essential and important control measure, and the system should be designed to minimize areas of stagnation and low flow. Care should be taken to ensure that any modifications to the system do not introduce areas of stagnation and low flow. Where such areas are unavoidable, design and operation should aim to at least reduce stagnation and low flow.

For example, around mixing valves, the outlet should be as close to the valve as possible. Commercially available thermostatic mixers can be fitted into the outlet to minimize the zone at risk of being colonized by bacteria.

Water systems at risk from stagnation should be periodically flushed or disinfected, and temperatures that are optimal for growth of *Legionella* should be avoided. However, where flushing is used, the likely exposure of people to aerosols generated during flushing must be considered.

Control of temperature

Keeping water temperature outside the ideal range for legionellae is an effective control measure for both hot and cold-water systems (Figure 3.3 shows the effects of temperature on survival and growth of *Legionella*).

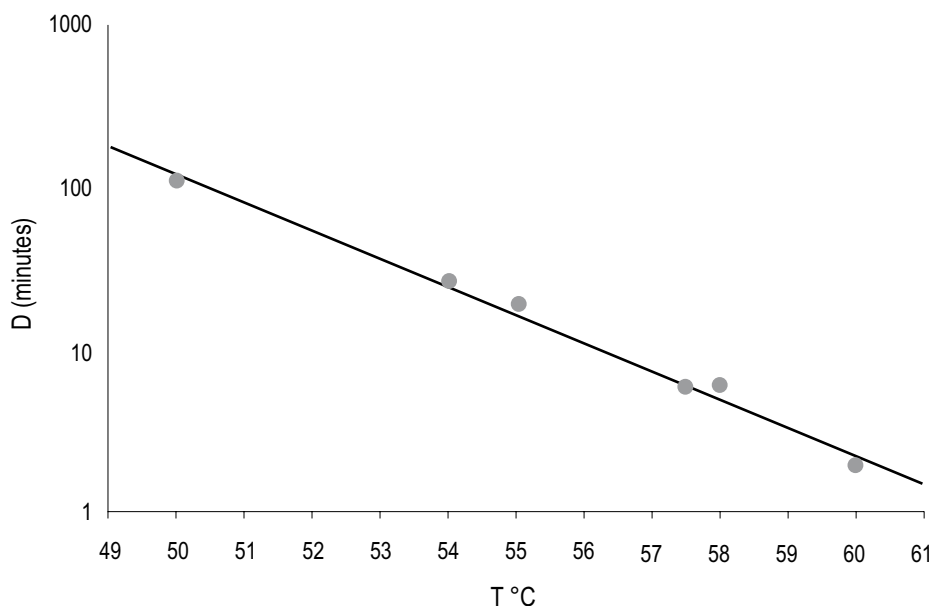
Water systems should:

- avoid water temperatures between 25 °C and 45 °C to prevent *Legionella* colonization
- ideally, maintain cold water below 20 °C
- ideally, maintain hot water above 50 °C.

In many systems (e.g. cooling towers and some cold and hot-water systems), maintaining these temperatures is not possible because of the nature of the system. Within such systems, temperatures should be maintained at the upper or lower limits of the *Legionella* multiplication range.

In domestic and public hot-water systems, control measures for reducing the proliferation of *Legionella* must not increase the risk of scalding, particularly for children, the elderly or people with disabilities.

Figure 3.3 Decimal reduction times for *L. pneumophila* serogroup 1 at different temperatures



Decimal reduction time (D) = time in minutes to kill 90% of the population of *Legionella*

Source: data combined from Dennis, Green & Jones (1984); Schulze-Robbecke, Rodder & Exner (1987)

Control of microorganisms

Controlling protozoa is critical in reducing the risk of legionellosis; currently, the best way to achieve this is to prevent the development of the biofilms on which the protozoa graze (Donlan, 2002). However, preventing biofilm development can be difficult, particularly in dynamic water systems such as cooling waters and spas, where the water flow is disrupted and large amounts of nutrients may enter.

Any strategy for microbial control depends on water chemistry, temperature and the use of the water system. Ideally, microbial control will be achieved using the control measures described above. However, this is often not the case because of the characteristics of different water systems. For example, some industrial water systems are never disinfected because the cooling systems are open to the environment and would quickly be reinoculated with microorganisms after disinfection. In some water systems, chemical control of *Legionella* may not be safe because of the system's design. Therefore, a chemical control strategy should take into account system design, operating parameters and water chemistry (including the potential for production of disinfection by-products).

A WSP should take into account the unique features of the individual water system to which it is to be applied. Microbial control strategies are unlikely to be effective if other control strategies, notably flow rates, temperatures and maintenance, are neglected.

Comparison of control methods

Table 3.2 summarizes the advantages and disadvantages of alternative methods of controlling *Legionella* in reticulated water systems and cooling towers.

Table 3.2 Advantages and disadvantages of alternative methods for controlling *Legionella* in piped water systems and cooling towers

Method	Advantages	Disadvantages
Keeping temperature <20 °C	• Simple, effective and easily monitored • Little significant growth of <i>Legionella</i>	• Only really applicable to drinking water systems
Keeping temperature >50 °C	• Simple, effective and easily monitored	• Does not eliminate legionellae • Requires circulation temperature to be near 60 °C • Difficult to maintain temperatures in old systems • Requires protection against scalding
Periodic flushing with hot water at 50–60 °C (usually an essential part of control by high temperature, above)	• Simple, effective and easy to monitor	• Not applicable in cold-water systems • Requires protection against scalding • Must be maintained and inspected to achieve consistent control • Recolonization occurs within days
Dosing with sodium hypochlorite	• Proven, effective disinfection technique • Simple to use • Relatively cheap	• Formation of trihalomethanes • Needs protection (e.g. carbon filter) for dialysis patients • Toxic to fish • Affects taste and odour • Not stable, particularly in hot water • Increases corrosion of copper
Dosing with monochloramine	• More persistent than chlorine • Simple to use in mains distributions • Penetrates into biofilms	• Needs protection (e.g. carbon filter) for dialysis patients • Toxic to fish • Affects rubber components • No commercial kit available for dosing small water systems
Dosing with chlorine dioxide	• Proven disinfection technique • Simple to use	• Formation of chlorite • Needs protection (e.g. carbon filter) for dialysis patients • Safety considerations (depending on method of generation)

Method	Advantages	Disadvantages
Dosing with hydrogen peroxide	• Simple to use	• Weak disinfectant • Suspected of mutagenicity
Copper and silver ionization	• Effective when prescribed concentrations are maintained	• Frequent monitoring of copper and silver needed • Pretreatment needed (pH, hardness) • Increased concentrations of copper and silver in water
Anodic oxidation	• Disinfection demonstrated	• Pretreatment needed (depending on effect of pH and hardness) • Effect on <i>Legionella</i> in biofilms not known
UV (ultraviolet) disinfection	• Proven disinfection technique • Simple to use	• Effective only at point of application; no control downstream (no residual) • Not suitable for turbid waters • No effect on biofilm formation
Ultrafiltration at point of entry to the building or system	• Physical disinfection barrier • Effective removal of biomass and particles	• No inactivation of <i>Legionella</i> downstream of the filter within system • Effect on formation of biofilms and sediment not known
Point-of-use filters	• Physical barrier • Easy to install (may require some modification of the outlet) • Suitable for hot and cold-water systems • Good for use in systems exposing high-risk patients	• Only suitable at point of use • Must be replaced regularly • Particulates in water may reduce flow and operational life • Expensive
Pasteurization heat with flushing	• Disinfection barrier • Useful as short-term remedial measure • Simple to apply in hot-water installation	• Transient effect on <i>Legionella</i> • No limitation of biofilm formation • Scalding risk
Non-oxidizing biocides	• Proven technique for cooling systems	• Not suitable for potable water systems • Most not applicable to spa pools • Resistant populations may develop • Need to alternate two different biocides • Often concentrations cannot be readily monitored • Difficult to neutralize for sampling purposes

Note: No indication of costs is given because costs depend on many local factors, including the complexity of the system involved.

The appropriate design of new water systems is a critical step in controlling *Legionella* proliferation. The control measures listed in Table 3.2 should be borne in mind when designing and constructing the system. Poor design and construction will inevitably compromise attempts to implement effective control measures, which in turn will have a serious impact on *Legionella* control.

Monitor control measures

A monitoring programme should be developed for the water system, to ensure that identified control measures are functioning effectively. Monitoring points should be identified throughout the system, for each control measure, on the basis of system design, operating parameters and high-risk areas. Particular attention should be given to areas where control is most difficult to achieve, and areas where *Legionella* is most likely to grow.

Monitoring of control measures should be primarily based on tests that are simple and rapid to apply (ISO 5667 (ISO, 2001) can be used as a guide in developing a sampling method); and where possible, monitoring equipment should be online and automatic. The equipment should also be set up in such a way that remedial action is instigated as soon as failures in control measures are detected, and before levels fall outside predetermined target ranges, such as those shown in Table 3.3 (each target must be a measurable parameter (e.g. temperature, biocide doses or heterotroph counts)).

Results from system monitoring should be used in assessing the maintenance programme and in improving the system. All monitoring records should be kept current and accessible, so that the system can be assessed.

Table 3.3 Examples of microbiological quality monitoring and action level specifications for cooling water systems

Aerobic heterotrophic count CFU/ml	Action required
10 000 or less	Acceptable control. No remedial action required.
More than 10 000 and up to 100 000	Review programme operation. The count should be confirmed by immediate resampling. If a similar count is found again, a review of the control measures and risk assessment should be carried out to identify remedial actions.
More than 100 000	Implement corrective action (action to be taken when the results of monitoring at the control point indicate a loss of control). The system should immediately be resampled. It should then be “shot dosed” with an appropriate biocide, as a precaution. The risk assessment and control measures should be reviewed to identify remedial actions.

Source: Adapted from HSE (2004)
CFU = colony forming units

Validate effectiveness of water safety plan

This step involves developing procedures to verify that the WSP is working effectively, and will meet the predetermined target; that is, it involves monitoring individual components of the water system to determine whether the WSP has effectively controlled *Legionella* in the system. Validation and verification are defined in Box 3.2.

Box 3.2 Verification and validation

Validation is the process of obtaining accurate and reliable evidence that a water safety plan is effective.

Verification is defined as the use of methods, procedures or tests, in addition to those used in operational monitoring, to determine whether the performance of the supply complies with the stated objectives outlined by the health-based targets. Verification might be undertaken through independent surveillance; it provides an indication of the overall performance of the system.

Source: WHO (2004).

If control of *Legionella* is found to be inadequate, the operational procedures should be reviewed and control measures re-evaluated as a matter of urgency. A health risk assessment of the system may be necessary, to determine whether the management contingency plan should be used (e.g. shot dosing the system with biocide).

There appears to be little correlation between *Legionella* culture test results and human health risk (Kool et al., 1999; Bentham, 2002). *Legionella* testing cannot be considered a control measure, because of:

- uncertainties about the reliability of culture
- time delays
- differences between culture requirements for different *Legionella* species
- dynamics of the population.

Chapter 11 provides more details on laboratory diagnosis of *Legionella*.

Although *Legionella* testing cannot be considered a control measure, it can be used in validation to provide some evidence that the WSP is effective and that control measures are operating properly. Validation normally includes more extensive and intensive monitoring than routine operational monitoring, and its aim is to determine whether system units are performing as assumed in the system assessment (see Section 3.3.1). Operational monitoring of control measures should be by measures that provide real-time results (e.g. monitoring of biocide concentrations, temperature and pH); sampling for *Legionella* cannot provide results sufficiently quickly to be useful in operational monitoring.

3.3.3 Management and communication

The steps involved in management and communication — shown in Figure 3.2, and discussed below — are to:

- develop supporting programs
- prepare management procedures
- establish documentation and communication procedures.

Develop supporting programs

Supporting programmes are actions that are important for ensuring water safety but are not control measures. They include:

- training and educating personnel involved in activities that could influence the water quality (these activities should include refresher training, to regularly assess and update competencies; also, records of all training should be maintained)
- gathering evidence-based data on which to base health-related targets
- developing verification protocols for the use of chemicals and other control measures (e.g. to ensure the use of suppliers that participate in quality assurance programmes).

Supporting programmes can be identified and incorporated within the WSP as a part of the system assessment process.

Prepare management procedures

Effective management of the water system should include procedures for:

- the actions that should be taken in response to variations in the water system that occur during normal operational conditions
- the actions that should be taken in specific “incident” situations
- the actions that should be taken in unforeseen and emergency situations.
- Procedures should be realistic, without increasing the complexity of the system’s operation. They should clearly identify the responsibilities of all people involved in system operation and maintenance, and should identify an individual responsible for overall implementation.

Establish documentation and communication procedures

Documentation of a WSP should include:

- details of all personnel involved in developing and identifying control measures and maintenance strategies (this list should also clearly identify an individual with managerial responsibility for implementing and reviewing the WSP)

- clear statements of responsibilities and a chain of communication between all these personnel to ensure:
 - effective cooperation between people engaged in system operation and maintenance
 - a rapid and multidisciplinary response to failures in the management strategy
 - coordination of a continuing review and evaluation process
- a list of identified targets, control measures and monitoring points
- all maintenance records, control measure monitoring data and control verification data
- actions to be taken as part of routine periodic maintenance of the system and its components, and interventions to be undertaken should monitoring or verification data suggest loss of control
- a reporting process that informs all involved people of system status and control, and identifies actions to be taken in reporting monitoring and verification results
- a contingency plan that clearly outlines actions to be taken and chains of communication and reporting in an emergency, and including a definition of the circumstances under which the contingency plan will be instigated
- a description and assessment of the water system, including a current schematic diagram of the system
- the plan for operational monitoring and verification of the system (e.g. frequency of monitoring, target levels for parameters)
- a description of supporting programmes (e.g. training targets and manuals)
- water safety management procedures for normal operation, incidents and emergency situations.

Records are essential for reviewing the adequacy of the WSP and for ensuring that the water system adheres to the WSP. The following records should be kept:

- supporting documentation for developing the WSP, including validation (the process of obtaining accurate and reliable evidence that the WSP is effective)
- records and results generated through operational monitoring and verification
- outcomes of incident investigations
- documentation of methods and procedures used
- records of employee training programmes.

Periodic review of the records is recommended, so that trends can be identified and appropriate actions taken to maintain the safety and quality of the water system.

A person involved in implementing the WSP should be responsible for risk communication. This person should also be responsible for developing a risk management plan (to effectively convey the WSP to all parties involved in the process, and to other entities where appropriate). The plan should clearly identify and interpret the goals of the risk assessment and the WSP; it should include:

- modes of communication to be used
- background information on the risk posed by *Legionella*, derived from the risk assessment and system assessment
- the goals of the WSP in addressing the risk posed by *Legionella*
- content and target audiences for communication
- sources of further information about the water system and *Legionella* contamination.

Communication strategies should include procedures for promptly advising stakeholders of any significant incident in the water system. This includes:

- notifying the public health authority
- making summary information available to the public
- establishing mechanisms for receiving and responding to community concerns.

The agencies responsible for monitoring should develop strategies for disseminating and explaining the significance of health-related information.

3.4 Surveillance

Surveillance is the systematic collection, orderly consolidation, and analysis of data to verify that health-based targets, system assessments and control measures are operating properly. It might include:

- internal audit and external audit (by the health department) to confirm that operational monitoring and corrective actions are being undertaken as stated in the WSP
- monthly heterotrophic colony counts at the tap and in the source water (to track trends and changes, rather than as an absolute indicator, and to be undertaken by an accredited laboratory)
- six-monthly sampling for legionellae in water at source and at the tap.

Chapter 4 Potable water and in-building distribution systems

Richard Bentham, Susanne Surman-Lee, John V Lee, Emmanuel Briand, Dick Van de Kooij

This chapter describes how a water safety plan (WSP) can be applied to assessing and managing the risks associated with *Legionella* in potable water and in distribution systems in buildings. It should be read in conjunction with Chapter 3, which discusses the different elements that make up a WSP, and shows how a WSP fits within the framework for safe water quality developed by the World Health Organization (WHO).

As explained in Chapter 3, a WSP has 10 steps that fit within the three main areas of system assessment, monitoring and management and communications (see Figure 3.2). A WSP must be comprehensive, and all 10 steps should be implemented in assessing and managing the risks associated with *Legionella*. However, this chapter focuses on parts of the WSP where information specific to potable water and in-building distribution systems is needed.

4.1 Background

The first published report of *Legionella* being transmitted through a potable water installation involved renal transplant patients who acquired the infection in a hospital (Tobin et al., 1980; see Chapter 6). Since then, *Legionella* has been observed in water systems in many different types of buildings, including hotels, homes and factories, and in ships (Bartlett et al., 1983; Habicht & Muller, 1988; Stout et al., 1992; Allen, Prempeh & Osman, 1999; Castellani Pastoris et al., 1999). *Legionella* has been found throughout engineered water systems, from the mains supply to consumers' taps. Once present in a water system, legionellae can be isolated from a range of sources, unless adequate controls are in place (Stout, Yu & Best, 1985; Colbourne & Trew, 1986).

Legionella numbers in a plumbing system are influenced by many factors, and may vary considerably in time and place, particularly in large, complex systems. Since legionellae can grow in association with many different microorganisms (see Chapter 2, Section 2.3), it is important to control other microorganisms to reduce the proliferation of legionellae.

Infection with *Legionella* requires both proliferation and exposure. Potable water systems containing *Legionella* are a significant cause of sporadic cases of legionellosis acquired in the community (Stout, Yu & Best, 1985; Yu, 1993; Venezia et al., 1994). Such systems are also the main cause of nosocomial infection (through aspiration or direct infection of wounds — Lowry & Tompkins, 1993), with cases reported in many European countries (e.g. see Box 4.1) and in North America.

Box 4.1 Cold-water tap as a source of fatal nosocomial *Legionella* pneumonia in a rehabilitation centre in the Netherlands

Hoebe et al. (1999) reported two fatal cases of legionellosis in a rehabilitation centre in the south of Limburg, the Netherlands. The water supply was investigated, and *Legionella* was cultured from:

- respiratory patients' specimens
- water samples and smears from all mixing taps used in showers
- samples from hot and cold-water taps from the infected ward and from the other wards.

The *L. pneumophila* (serotype 1) found in the water supply was the same as that cultured from the sputum of the two male patients who died of legionellosis.

The cold-water pipes ran alongside both the hot-water pipes and the central heating system, and the circulating cold water sometimes reached 40 °C, which is within the growth range of *Legionella*. Also, the infected ward was closed during weekends, meaning that the water remained stagnant.

The study's authors concluded that multiplication of *Legionella* in the water supply was probably stimulated by the combination of an elevated cold-water temperature and the regular stagnation of water.

In northern Europe, about 50% of cases of legionellosis are associated with travel, and the infection is often associated with hotel water systems (Joseph et al., 1998). *Legionella* has also been isolated from water installations in domestic premises; for example, in a study of sporadic cases of legionellosis in the United Kingdom, legionellae were isolated from approximately 15% of the homes of infected patients but from only about 5% of homes tested as controls (Coward et al., 1999).

4.2 Water safety plan overview

A WSP needs to be comprehensive; however, an overview of such a plan is shown in Table 4.1, as an example of the type of information a plan might contain. As explained in Chapter 3, a WSP is part of a framework for safe water quality that also includes health-based targets and surveillance.

In the case of the sample WSP shown in Table 4.1, a health-based target for drinking water might be "Where possible, *Legionella* should be non-detectable". Table 4.2 gives examples of health-based targets for *Legionella*. The water quality in health-care facilities needs special attention, determined by the susceptibility of the patients; patients undergoing a severe immunosuppressive therapy (e.g. organ transplant or cancer therapy) are particularly at risk of infection.

Further information on health-based targets and information on surveillance for *Legionella* can be found in Sections 3.2 and 3.4 of Chapter 3, respectively.

The remainder of this chapter provides information relevant to a WSP specific for potable water and in-building distribution systems, for each of the three main areas of a WSP:

- system assessment (Section 4.3)
- monitoring (Section 4.4)
- communication and management (Section 4.5).

Sections 4.3–4.5 should be read in conjunction with Section 3.3 from Chapter 3.

Fundamentally, the responsibility for managing the risk of legionellosis belongs to the owner or manager responsible for the potable water or in-building distribution system. To ensure that the WSP is properly implemented, the owner or manager should assign tasks, ensure that documentation is complete and current, and hold people accountable.

Table 4.1 Example of a water safety plan for potable water and in-building distribution systems

Process step	Water source and receipt	In building			
		Storage	Distribution	Hot water	Consumer
Assess hazards and prioritize risks (example) ↓	Low disinfection residual leading to presence of legionellae in received water	Elevated temperature causing proliferation of legionellae	Entry of nutrients through sullage (grey-water), sewage, etc.), providing growth source for legionellae	Temperatures of 25–50°C, leading to proliferation of legionellae	High-aerosol generating devices causing potential for inhalation of legionellae
Identify control measures (example) ↓	Water supplier to meet health-based water standards Water guidelines to be based on national guidance and/or liaison with the health department	Temperature to be below 25°C for cold-water storage	Backflow to be prevented	Minimum flow temperature of 60°C to be maintained in water leaving the heating unit, and of 50°C at the tap (1 minute after leaving the heating device)	No high-aerosol generating devices to be in place after two years (to be replaced by low-aerosol generating devices)
Monitor control measures (example) ↓	Agreement between water authority and user; legionellae levels in source water to be checked periodically	Plumbing staff to check temperature monthly by thermometer and surface probe	Plumbing staff to check backflow prevention devices annually	Plumbing staff to check temperature monthly by thermometer and surface probe at “sentinel” points	Point-of-use treatment unit agreement with contractors; building maintenance supervisor to oversee contract and audit every 6 months

Process step	Water source and receipt	In building			
		Storage	Distribution	Hot water	Consumer
Prepare management procedures (example)	Water authority to immediately communicate any deviations in agreed water quality to user and to health department	Storage tank to be isolated and temperature problem addressed	Backflow prevention devices to be replaced if not working; system to be super-chlorinated; communication protocol to be followed	Water source to be isolated if possible and source disinfected; and temperature problem addressed	Water source to be isolated if possible and source disinfected
Develop supporting programmes (example)	Staff training and education; maintenance and calibration; backflow and plumbing controls				

Table 4.2 Examples of health-based targets for *Legionella* in piped water systems

Country	Value (CFU/litre)	Comment	Reference
France	<1000	• Target for general public facilities	Ministère de la Santé et des Solidarités (2005)
	<100	• Target for prevention of nosocomial infections	
	<50	• Target where at-risk patients are hospitalized	
Germany	1000		DVGW (2004)
The Netherlands	100	• Guideline target	VROM (2002)
United Kingdom	<100	• Guideline target	HSE (2004)

CFU = colony forming units

4.3 System assessment

This section should be read in conjunction with Section 3.3.1 of Chapter 3. The steps involved in system assessment, some of which are discussed further below, are to:

- assemble a team to prepare the WSP
- document and describe the system (Section 4.3.1)
- assess hazards and prioritize risks (Section 4.3.2)
- assess the system.

4.3.1 Document and describe the system

In documenting and describing the system, all relevant information and documentation should be compiled. Box 4.2 lists the particular components of a potable water distribution system that should be assessed.

Box 4.2 Components of potable water distribution system to be assessed

Particular components of a potable water distribution system that should be assessed include:

- the quality of water entering the system
- the design and construction of equipment (including operational information about temperature regime and water circulation)
- treatments (e.g. anticorrosion, antiscaling and disinfection) and timing of treatments
- systems, system components and equipment that have the potential to generate aerosols
- the temperature of storage tanks and the environment in which the system is located (both in buildings and outside), including the location of the system network (e.g. pipes in conduits, ceilings, walls and floors)
- the periods of water use; for example, on a daily or weekly basis (e.g. sports facilities may use water on a weekly basis)
- the turnover of water in areas such as storage tanks
- the population using the system, including any particularly susceptible people
- the management structure
- the competence of personnel responsible for the system.

Potential exposure pathways and the proliferation of *Legionella* should be taken into account at the design stage, because modifying existing facilities can be complicated and expensive.

Once a desktop review of the system has been completed, a sanitary survey or “onsite” survey should be carried out to verify the system (see Chapter 4 of WHO, 2004).

4.3.2 Assess hazards and prioritize risks

This step involves collecting and evaluating information on hazards and conditions leading to their presence, to decide which are significant for safety and therefore should be addressed in a safety plan.

In assessing hazards, it is reasonable to assume that all water supply systems have the potential to become seeded with microorganisms, including legionellae, during construction, repair and maintenance, even if the water is treated. Legionellae are widespread in surface water, and numbers of *L. pneumophila* ranging from 10^4 to more than 10^7 cells/litre have been observed by direct immunofluorescence assay (Chapter 11).

In many cases, direct methods of molecular detection have shown *Legionella* to be present in drinking-water leaving treatment facilities and in distribution systems. Detection of low numbers of culturable *Legionella* is difficult; therefore, information about the presence and behaviour of the organism in distribution systems is scarce. Nevertheless, *Legionella* found in plumbing systems has frequently been shown to originate from drinking-water. Thus, it appears that *Legionella* may be present in distribution systems (at least in temperate climate zones), but at levels below the detection limit of culture techniques. There is no evidence that such low levels of contamination pose a direct health threat to consumers.

In assessing piped water systems, it is important to investigate whether the combination of factors present in the system is likely to lead to the proliferation of legionellae. Such factors are listed in Box 4.3 and discussed below. These factors are strongly interrelated, and it is not currently possible to rank them. The risk factors discussed below include not only those for growth of *Legionella*, but also those — such as aerosol production — that are likely to increase the risk of infection.

Box 4.3 Risk factors for growth of or exposure to *Legionella* in piped water systems

Factors that can lead to proliferation of, or exposure to, *Legionella* in piped water systems include:

- poor water quality and treatment failures
- distribution system problems such as stagnation and low flow rate
- construction materials that contribute to microbial growth and biofilm formation
- inefficient or ineffective disinfection
- water temperature of 25–50 °C
- presence of biofilms
- aerosol production.

Water quality and treatment — risk factors

As discussed in Chapter 2 (Section 2.3), *L. pneumophila* growth can only be sustained in piped water if nutrients are available, either from the source water or (directly or indirectly) from other microorganisms (Anand et al., 1983; Stout, Yu & Best, 1985, 1992; Barbaree et al., 1986; Vickers et al., 1987; Lück et al., 1991). Thus, poor quality water or water that has not been effectively treated may allow legionellae to proliferate within the system.

Distribution system — risk factors

Proliferation of legionellae is promoted by stagnation, which occurs, for example, in the deadends of distribution system pipework, and in storage tanks and systems that are not frequently used.

Another risk factor associated with potable water distribution systems is the potential dissemination of legionellae through aerosols. In the home, inhalation of legionellae can occur from the aerosols that are generated by showers and toilet flushing, and from devices such as nebulizers if they are cleaned or filled with tap water.

Construction materials — risk factors

In the past, water supply systems were generally constructed of metallic materials such as cast iron, galvanized iron, brass or copper. Metallic plumbing materials are increasingly being replaced with synthetic materials such as polyvinyl chloride (PVC) and polybutylene. These different construction materials vary in their potential to support microbial growth and biofilms. For example, synthetic materials may leach organic compounds that may provide a source of nutrients for microorganisms subsequently colonizing them (Colbourne & Ashworth, 1986), and copper is more resistant to colonization than synthetic materials; however, metallic materials are more prone to corrosion and this can encourage biofilm formation.

Certain natural materials such as hemp and natural rubber components promote biofilm formation and thus promote the growth of legionellae more than metallic materials, both in laboratory conditions and in practice (Niedeveld, Pet & Meenhorst, 1986). Hemp is a traditional jointing compound, and natural rubber components are often present (together with plastic materials) in pressure compensating vessels, and in flexible tubes and shower hoses.

Accumulation of sludge, scale, rust, or algae or slime deposits in water distribution systems supports the growth of *Legionella* (WHO, 2004).

Despite its natural resistance to biofilm formation, copper pipework can become corroded through biodeterioration, mediated by microorganisms. This is a particular problem in areas with soft water (Keevil et al., 1989). In some cases, dosing regimes with chlorine-based biocides have led to the failure of plumbing systems, requiring costly replacement (Keevil et al., 1989; Grosserode et al., 1993). The risk of colonization, therefore, should be balanced with other risks linked to the choice of materials, such as dissolution, corrosion and scaling.

Disinfection — risk factors

Chemical disinfection may not be effective against *Legionella* that are found in protozoa (Kilvington & Price, 1990). In addition, the complexity of many piped water systems, particularly in old buildings, makes effective disinfection difficult; for example, booster disinfection may not be effective in a complex system.

Presence of biofilms — risk factors

Bacteria in drinking-water systems tend to adhere to surfaces and develop an organic protective matrix, creating microenvironments known as biofilms (discussed in Chapter 2, Section 2.3). Legionellae can thrive in biofilms, either directly or as parasites of certain protozoa that graze on the films.

Temperature — risk factors

Risks from legionellae may be greater in warmer regions (subtropical and tropical), because temperature is an important factor in the ability of the microorganism to survive and grow. Published information about *Legionella* concentrations in drinking-water distribution systems

in warmer regions appears to be lacking, but up to 10^8 cells/litre have been found in surface waters in tropical regions, and *Legionella* has been cultured in high numbers from warm water sources (Ortiz-Roque & Hazen, 1987).

Naturally occurring *L. pneumophila* can survive and multiply in water at temperatures of 25–45 °C, with an optimal range of 32–42 °C and the greatest increase in viable counts at 37–42 °C (Yee & Wadowsky, 1982). The multiplication rate decreases at temperatures below 37 °C, with no observable growth below 20 °C (HSE, 2004). In certain geographical regions, temperatures may routinely be above 20 °C and, in some cases, may reach optimal temperatures for legionellae growth.

4.4 Monitoring

This section should be read in conjunction with Section 3.3.2 of Chapter 3. The steps involved in monitoring, some of which are discussed below, are to:

- identify control measures (Section 4.4.1)
- monitor control measures (Section 4.4.2)
- validate effectiveness of the WSP.

4.4.1 Identify control measures

This section should be read in conjunction with Table 3.2 of Chapter 3, which provides information on the advantages and disadvantages of alternative methods of controlling *Legionella* in piped water systems.

The focus of attention in managing legionellae risks should be on preventing both proliferation and exposure, in line with the multiple-barrier approach that forms part of a WSP. Systems will need to be assessed individually, and any treatment will need to be validated by testing for its effectiveness against legionellae and for the presence of legionellae in operating systems. For example, in order to choose appropriate control measures, it will be necessary to know the “normal” operating temperature of the water supply.

Water quality and treatment — control measures

Water from the supplier should meet the appropriate drinking-water standards or guidelines of the jurisdiction (e.g. WHO, 2004), and should not contain high levels of nutrients.

Measures for reducing numbers of *Legionella* are not routinely applied in drinking-water distribution systems because (as explained in Section 4.3) levels of *Legionella* are usually below the detection limit of culture techniques. However, in most countries, surface water treatment includes a series of barriers to eliminate or inactivate pathogenic microorganisms of faecal origin. These physical techniques, such as coagulation–sedimentation, filtration and disinfection will also reduce the number of legionellae (Kuchta et al., 1983).

Where temperature controls (discussed below) cannot be maintained, an alternative means of control needs to be implemented; for example, where legionellae multiply in warm areas of cold-water systems. The effectiveness of control measures for *Legionella* depends on many variables. Physical systems such as ultraviolet (UV) and filtration may be satisfactory if fitted near the point of use, but they are not dispersive; that is, they do not form a residual level of treatment throughout the water system and therefore will not affect biofilms harbouring *Legionella* downstream of their point of use. Criteria for a universal acceptable level of effectiveness have not been defined, but might include a required log reduction of *Legionella* in water and an effect on biofilms (e.g. reduction of formation or growth of biofilms).

Applying alternative control techniques requires detailed consideration of the extent and complexity of the system, and of the composition of the water. Where alternative measures are implemented, monitoring is needed to ensure that controls are adequate and maintained (see Section 4.5).

Tap diffusers reduce water use but can increase aerosol production. Therefore, in high-risk areas, such as hospitals, diffusers should not be installed (and facilities should consider removing diffusers that are already in place). Mixing valves should be as close to the shower outlet as possible, and shower fittings should be detachable so that they can be routinely cleaned and disinfected.

Distribution systems — control measures

Control of legionellae should begin at the design stage of the water system. There are many different designs for modern plumbed water systems that supply hot and cold water in buildings. Systems may be gravity fed, with a storage tank for cold water fed by the mains supply, or they may be pressurized, with no intermediate storage tank. Hot water is supplied from a water heater, calorifier, boiler or plate heat exchanger, depending on the scale of the system. Cold water is distributed either directly from the mains supply or via a cold-water storage tank.

Pipes should be as short as possible. In complex systems, regulating valves should be used to control flow. Deadends should be avoided in both the design and construction phases, and in existing systems they should either be removed or regularly flushed.

Standard system fittings should include devices to prevent backflow on heat production systems, and purge valves to prevent scaling and corrosion and facilitate monitoring. These should be installed at appropriate locations in the system, according to national standards.

Construction materials — control measures

The materials used to construct piped water distribution systems should be compatible with the chemical quality of water (after a corrective treatment) and should minimize bacterial growth.

Disinfection — control measures

To control *Legionella* numbers in the distribution system, a disinfectant residual should be maintained. Monochloramine residual (currently available only for mains distribution systems) appears to be effective against *Legionella* in biofilms, and may be more effective than chlorine (Kool et al., 1999).

Biofilms — control measures

A critical objective of any strategy to prevent the proliferation of *Legionella* in plumbing systems should be to minimize the development of biofilms hosting *Legionella*.

Routine cleaning of storages and control of nutrients in source water will reduce nutrient load and so help to reduce biofilm formation and growth.

Temperature — control measures

Temperature is critical in *Legionella* control. Consequently, water temperature should, as far as possible, be measured and registered.

Control measures for water temperature include the following:

- For recirculating hot-water systems, the temperature of the water leaving the heater should be not less than 60 °C and the temperature of the return should be not less than 50 °C. Very small differences between the temperature at the outlet of the heater and the returning water may indicate shortcuts in the circulation.
- For non-circulating hot-water systems without storage tanks, the length of the pipes connecting the heating device with the taps should be as short as possible.
- The temperature of hot water at the tap should reach its maximum value within one minute, and the temperature of the cold water within two minutes (HSC, 2000).
- The temperature of hot water reached within one minute at the tap should not be less than 50 °C, except where thermostatic mixer valves are installed.
- The temperature of cold water at the tap should not exceed 25 °C. Where possible, the temperature should be less than 20 °C, to reduce growth of legionellae. Where cold-water supplies are routinely above 20 °C, the water should be treated as a warm water supply.
- Where fail-safe thermostatic mixer valves are installed, the cold-water temperature should not exceed 25 °C and the hot should not exceed 50 °C immediately before the valves.
- Temperature increases of cold-water pipes, reservoirs and treatment devices should be prevented by appropriate insulation and sufficient distance between cold pipes and hot-water pipes or heating equipment.
- In systems in which water temperature at the tap cannot be maintained at 50 °C because of the risk of scalding a susceptible population (e.g. in an old people's home), alternative means of control should be implemented. Alternative measures include the use of biocides or periodic flushing (superheating) of the system with a return (and tap water) temperature of at least 60 °C. This measure requires stringent safety measures to prevent scalding.

4.4.2 Monitor control measures

This step involves defining the limits of acceptable performance and how these are monitored. Routine monitoring of a potable water distribution system will include surrogate observations, such as:

- turbidity
- disinfectant residual
- copper and silver ions
- structural integrity of the system
- temperature.

A thermometer and a surface probe are useful for measuring water temperatures at each part of a system, at outlets representative of the “worst case scenario” (i.e. at the points at which the risk is likely to be highest). Such outlets — often termed “sentinel points” — might include the furthest point from the water heater in a hot-water system, or the incoming water in a cold-water system.

The results of tests such as those listed above allow corrective actions (discussed in Section 4.5.1, below) to be taken to protect public health.

Tests carried out for legionellae and heterotrophic colony counts in the distribution system do not give timely information on the performance of the system, and are therefore most useful in validation and verification.

4.5 Management and communication

This section should be read in conjunction with Section 3.3.3 of Chapter 3. The steps involved in management and communication are to:

- develop supporting programs
- prepare management procedures (Section 4.5.1)
- establish documentation and communication procedures (Section 4.5.2).

4.5.1 Prepare management procedures

This step involves preparing management procedures, including corrective actions, for normal and incident conditions. Corrective actions include repairing defects, and possibly re-treating or discarding water that might be contaminated, to ensure that unsafe water is not supplied. Table 4.3 gives examples of values used as levels to trigger corrective action for *Legionella* in piped water systems in different countries. These values are generally used to support risk assessment or to monitor the effects of control measures.

Table 4.3 Examples of values used as levels for corrective action for Legionella in piped water systems

Country	Value (CFU/litre)	Comment	Reference
The Netherlands	>1000	• Immediate action is needed to prevent closure of (part of) system involved	VROM (2002)
United Kingdom	100–1000	• Action depends on whether just one or two or the majority of samples are positive; review of control measures and risk assessment required; possible disinfection	HSE (2004)
	>1000	• Immediate review of control measures and risk assessment required; possible disinfection	
United States	>10 000	• Prompt cleaning and/or biocide treatment of the system	OSHAHD (2005)
	>100 000	• Immediate cleaning and/or biocide treatment; take prompt steps to prevent employee exposure	

CFU = colony forming units

4.5.2 Establish documentation and communication procedures

Table 4.4 gives an example of documentation for monitoring and corrective action.

Table 4.4 Example of documentation for monitoring and corrective action

Process step	Indicator	Monitoring		Operational limits	Corrective actions	
Heating of water	Temperature	What	Temperature	Outlet: Not less than 65 °C Return: Not less than 63 °C	What	Improve water circulation and/or increase water temperature
		How	Thermometer or thermocouple data logger		How	Add extra pump on the return to the water heater Turn up thermostat on calorifier
		When	Daily or online		When	Immediately
		Where	Return to water heater and at the outlet of the heater		Who	Plumber (for pump) Building engineer (for calorifier)
		Who	Building engineer			

Chapter 5 Cooling towers and evaporative condensers

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This chapter describes how a water safety plan (WSP) can be applied to assessing and managing the risks associated with *Legionella* in cooling towers and evaporative condensers.

It should be read in conjunction with Chapter 3, which discusses the different elements that make up a WSP, and shows how a WSP fits within the framework for safe water quality developed by the World Health Organization (WHO).

As explained in Chapter 3, a WSP has 10 steps that fit within the three main areas of system assessment, monitoring and management and communications (see Figure 3.2). A WSP must be comprehensive, and all 10 steps should be implemented in assessing and managing the risks associated with *Legionella*. However, this chapter focuses on parts of the WSP where information specific to cooling towers and evaporative condensers is needed.

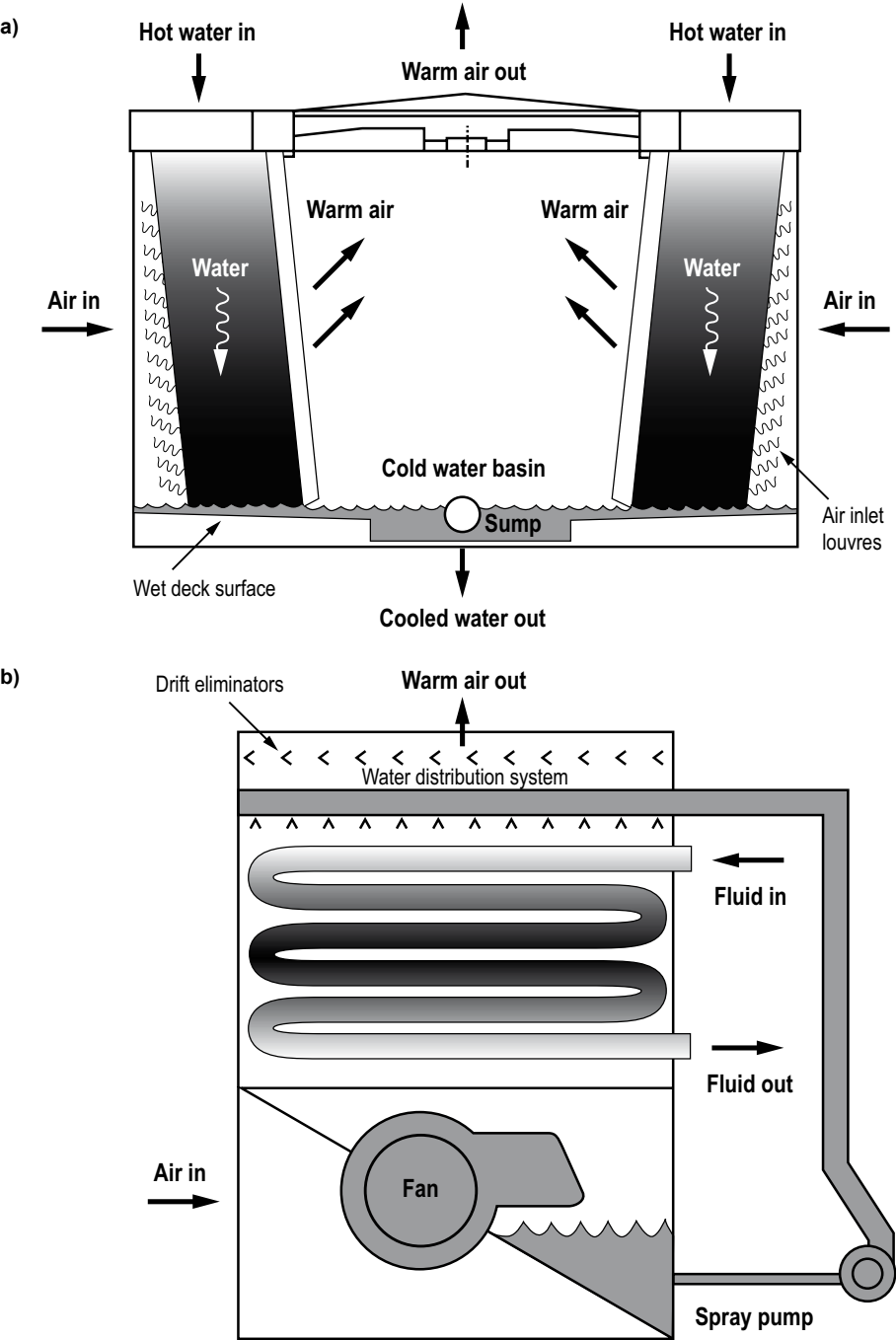
5.1 Background

Cooling towers and evaporative condensers (also known as evaporative fluid coolers or closed-circuit cooling towers) are heat-transfer devices in which warm water is cooled by evaporation in atmospheric air (see Figure 5.1). These devices are used:

- to provide cooling for a wide variety of industrial processes
- for refrigeration plant used in cold stores
- to cool water for air-conditioning to buildings.

Air movement through the tower or condenser is produced by fans or, occasionally, by natural convection. Aerosols generated by the operation of cooling towers and evaporative condensers can transmit legionellae to susceptible hosts (Broadbent, 1996; Geary, 2000).

Figure 5.1 Configuration of typical cooling towers and evaporative condensers



(a) Typical cross-flow cooling tower
(b) Typical counterflow closed-circuit evaporative condenser

Source: artwork courtesy of Baltimore Aircoil Co.

5.1.1 Cross-flow cooling towers

As shown in Figure 5.1(a), cooling towers are heat exchangers; they act by cooling water that is in direct contact with the air moving through the tower. Most towers use a medium, referred to as “fill” or “pack”, to maximize the surface area of water in contact with air and therefore available for evaporation.

Water from the cooling tower is piped from the tower to a condenser (or other heat source), where it is heated. Warm water is distributed via a spray, or a trough and gutter system at the top of the tower, and falls down over the fill against the countercurrent of air. The warm water is then piped back to the cooling tower to be cooled, and the process is repeated. There may be tens or even hundreds of metres of piping between the tower and the point where the source is cooled. The piping circuit can be quite complex in some industrial settings, where several devices may be cooled.

5.1.2 Counterflow evaporative condensers and cooling towers

As shown in Figure 5.1(b), counterflow evaporative condensers and cooling towers are similar to cross-flow cooling towers, except that the warm fluid that is being cooled is contained inside a tubular matrix and does not come into direct contact with the air.

The water has only a short circuit from the sump at the base to the distribution system at the top. It then flows down over the tube bundle, in the opposite direction to the airflow, thus cooling the fluid within the tubes. Figure 5.1(b) shows how vapour enters and liquid exits the condenser coil.

5.1.3 Links to outbreaks of legionellosis

Cooling towers and evaporative condensers have been implicated in many outbreaks of legionellosis. As discussed in Chapter 1, *L. pneumophila* serogroup 1 MAb2-reactive strains are the primary legionellae associated with outbreaks of disease from these systems. The causative organism has been readily isolated from many of these devices, usually as a result of neglect or insufficient maintenance (Fields, Benson & Besser, 2002), as illustrated by the example given in Box 5.1. A significant proportion of outbreaks of legionellosis have been attributable to the start-up of stagnant systems without adequate chemical treatment (Bentham & Broadbent, 1993).

Box 5.1 An outbreak of legionellosis at the Melbourne Aquarium, April 2000

Between 11 and 27 April 2000, the Melbourne Aquarium, Australia, was linked to 125 confirmed cases of legionellosis. The cases were caused by *Legionella pneumophila* serogroup 1. Two case-control studies confirmed the source of the outbreak and investigated risk factors for infection. The aquarium cooling towers were found to be poorly disinfected and contaminated with *L. pneumophila*, and visiting the aquarium was significantly associated with disease. The case-control studies indicated that current smoking was a dose-dependent risk; in contrast, chronic illness and duration of exposure at the site were not significant risks (Greig et al., 2004).

The number of cooling towers in existence globally is not known, but about 30 000 are registered in the United Kingdom alone. To date, large-scale natural updraft towers, such as those commonly associated with electricity generation, have not been implicated in outbreaks of legionellosis, although the potential for their involvement cannot be dismissed.

The air exhausting from cooling towers and evaporative condensers carries two types of water:

- water vapour that has evaporated within the device, which may recondense and appear as steam
- water droplets that have been generated within the device and carried in the airflow; if carried over without initial evaporation, these droplets are termed “drift”.

The water droplets in drift will contain any dissolved salts or suspended particles, including organisms that were in the original water. It is these droplets that can create an infectious aerosol when the water evaporates in the open air outside the tower, unless appropriate controls are in place.

5.2 Water safety plan overview

A WSP needs to be comprehensive; however, an overview of such a plan is shown in Table 5.1, as an example of the type of information a plan might contain. As explained in Chapter 3, a WSP is part of a framework for safe water quality that also includes health-based targets and surveillance.

Most cooling towers and evaporative condensers are likely to become contaminated with *Legionella* at some point in their serviceable life (Koide et al., 1993; Bentham, 2000). It is unrealistic to try to prevent entry of the organism into the cooling tower or to create an environment that entirely precludes its growth and multiplication, although this is desirable. In the case of the sample WSP shown in Table 5.1, a health-based operational target might be to control microbial growth through modification of the environment and the use of

water treatments (including chemicals and antimicrobials). The water quality in health-care facilities needs special attention, determined by the susceptibility of the patients; patients undergoing a severe immunosuppressive therapy (e.g. organ transplant or cancer therapy) are particularly at risk of infection.

Further information on health-based targets and information on surveillance for *Legionella* can be found in Sections 3.2 and 3.4 of Chapter 3, respectively.

The remainder of this chapter provides information relevant to a WSP specific for cooling towers and evaporative condensers, for each of the three main areas of a WSP:

- system assessment (Section 5.3)
- monitoring (Section 5.4)
- communication and management (Section 5.5).

Sections 5.3–5.5 should be read in conjunction with Section 3.3 from Chapter 3.

Cooling towers and evaporative condensers are often designed simply to optimize heat transfer and thermal efficiency, and the practices described here might not be included in typical water treatment programmes for such devices. However, an effective water treatment programme that reduces the risk of legionellosis and thus ensures safer operation of the system also leads to more efficient operation (because there is less fouling) and longer system life (because there is less corrosion) (Broadbent, 1996).

Fundamentally, the responsibility for managing the risk of legionellosis belongs to the facility owner or manager. To ensure that the risk management plan is properly implemented, the owner or manager should assign tasks, ensure that documentation is complete and current, and hold people accountable.

Table 5.1 Water safety plan overview — cooling towers and evaporative condensers

Process step	Water source	Heat exchanger	Distribution	Cooling tower
Assess hazards and prioritize risks (example) 	High nutrients and microbial load in source water	Elevated temperature and nutrients in biofilm, causing proliferation of legionellae	Stagnant water in deadlegs (areas of little or no flow) in the pipework, resulting in proliferation of legionellae	Excessive drift loss from the tower exhaust, disseminating aerosols, and potentially legionellae, into the community
Identify control measures (example) 	Routine disinfection of water at 0.5 mg/l free residual chlorine	Routine cleaning of the heat exchanger Operate condenser at minimum operating temperature Chlorine 0.5 mg/l free residual	Treated water (chlorine 0.2–0.5 mg/l free chlorine residual and corrosion inhibitors) through the system	Well-fitted and designed drift eliminators ^a
Monitor control measures (example) 	Chlorine online (with chlorine/redox probe) Turbidity online	Chlorine and temperature online	Routine review of process diagram (desktop and onsite) to identify areas of concern or stagnation	Inspect drift eliminators monthly; look for drops and “splashouts” around the eliminators
Prepare management procedures (example)	Point-of-use filtration and disinfection programme; possible treatment for dissolved solids	Shut down condenser; drain and implement physical cleaning and disinfection protocol	Remove deadlegs where possible	Regularly replace drift eliminators
Establish verification and surveillance (example)	- Internal audit and external audit (by the health department) to confirm that operational monitoring and corrective actions are being undertaken as stated in the WSP - Monthly heterotrophic colony counts in the system and in the source water (to track trends and changes, rather than as an absolute indicator, and to be undertaken by an accredited laboratory)			
Develop supporting programmes (example)	Staff training and education; maintenance and calibration			

^a Drift eliminators are inertial stripping devices that are used to remove water droplets.

5.3 System assessment

This section should be read in conjunction with Section 3.3.1 of Chapter 3. The steps involved in system assessment, some of which are discussed further below, are to:

- assemble a team to prepare the WSP
- document and describe the system (Section 5.3.1)
- assess hazards and prioritize risks (Section 5.3.2)
- assess the system.

5.3.1 Document and describe the system

In documenting and describing the system, all relevant information and documentation should be compiled. Box 5.2 lists the particular components of a potable water distribution system that should be assessed.

Box 5.2 Components of cooling towers and evaporative condensers to be assessed

Particular components of cooling towers and evaporative condensers that should be assessed include:

- the quality of water entering the system
- the design of the devices and the distribution system
- nutrient sources
- the population using the system, including any particularly susceptible people
- the management structure
- the competence of personnel responsible for the system.

Once a desktop review of the system has been completed, a sanitary survey or “onsite” survey should be carried out to verify the system (see Chapter 4 of WHO, 2004).

5.3.2 Assess hazards and prioritize risks

This step involves collecting and evaluating information on hazards and conditions leading to their presence, to decide which are significant for safety and therefore should be addressed in a safety plan.

Source water quality — risk factors

The make-up water for a cooling tower or evaporative condenser will usually come directly from a municipal or well supply. However, sometimes a holding tank is used, which may contain rust, sludge and sediment. In some very large systems, it may be necessary to use surface water from lakes, rivers, streams, or reservoirs as make-up water; such sources are usually laden with microorganisms and nutrients from the environment.

Water treatment — risk factors

In the dynamic environment of a cooling tower system, water treatment chemicals do not perform in the same way as they do in a controlled laboratory trial (England et al., 1982). Also, the temperature and flow velocities of cooling tower water will vary at different locations within the system. Many other parameters, such as pH, conductivity, total dissolved solids, suspended matter and the biological mass within the system, can vary over a relatively short period, affecting water treatment.

Disinfection — risk factors

Efficacy of disinfection depends on water quality parameters such as pH and turbidity, which may compromise the disinfection process.

Applied microbial control programmes never sterilize cooling water systems. Even if enough chemical or other agent could be added to achieve sterilization, the system would rapidly become recolonized with microorganisms, since cooling systems are open to the environment. The most significant practical consequence of attempted sterilization would be selection in biofilms of increasingly tolerant microbial communities comprising the survivors of the applied antimicrobial treatment (Russell, 2000).

Biofilms — risk factors

Cooling towers and evaporative condensers typically move large quantities of air, and are excellent air scrubbers or washers. Thus, dirt, dust and other particulate matter enter the cooling tower water in the evaporative cooling process, as large amounts of air are moved through the unit. Depending on location, the quantity of such material added to the cooling water can be substantial (e.g. several kilograms per day).

Organic matter and other debris present in the air can therefore accumulate in the cooling water. This material may serve as a nutrient source for the growth of microorganisms, including legionellae. Diverse biofilms, which can support the growth of legionellae, may be present on all wet or moist surfaces throughout the system; for example, on heat exchangers, the fill, the sump and pipes (Geary, 2000; Donlan, 2002).

Temperature — risk factors

The typical temperature of the water in a cooling tower ranges from 29 °C to 35 °C at the heat exchanger, and from 22 °C to 28 °C at the cooling tower. These temperature ranges are conducive to the growth of legionellae and their hosts.

Design and materials used in construction — risk factors

Stagnation of the system or areas of stagnant water (e.g. deadlegs) prevent proper chemical treatment of the system, and allow legionellae and their hosts to proliferate.

Spray drift — risk factors

Even with appropriate design and under normal operation, some water droplets that are small enough to be inhaled (i.e. $<5\ \mu\text{m}$ in diameter) can leave the drift eliminator. Also, some larger droplets leaving the unit may be reduced to $5\ \mu\text{m}$ or less by evaporation (Guideline 12–2000 in ASHRAE, 2000).

Wherever possible, cooling towers should be located well away from building air intakes, other building openings and areas of public access. The influence of adjacent buildings, as well as prevailing wind directions, should be taken into account when locating a cooling tower. Consideration should be given to the effects of reversal of airflow through some towers when the tower fan is idle, and preventive dampers should be installed if necessary. In certain situations, the potential risk of having a tower in a particular site may be so great as to require its relocation; for example, where there are air inlets to hospital wards with high-risk patients.

Dry cooling systems are used in some situations, particularly on small (175–350 kW) systems. Although such systems use substantially more energy, and are typically larger and noisier than cooling towers, there is no known *Legionella* risk associated with dry systems.

5.4 Monitoring

This section should be read in conjunction with Section 3.3.2 of Chapter 3. The steps involved in monitoring, some of which are discussed below, are to:

- identify control measures (Section 5.4.1)
- monitor control measures (Section 5.4.2)
- validate effectiveness of the WSP.

5.4.1 Identify control measures

The overall goal of water treatment for cooling towers and evaporative condensers is to provide a heat-transfer fluid that allows equipment to function optimally and use water efficiently. This is achieved by minimizing microbial growth, scale, corrosion, and sediment or deposition of solids (organic or inorganic) on heat-transfer surfaces, through implementing the control measures outlined below.

The focus of attention in managing legionellae risks should be on preventing both proliferation and exposure, in line with the multiple-barrier approach that forms part of a WSP.

Source water quality — control measures

Where a holding tank is used to hold make-up water, the tank should be cleaned of rust, sludge and sediment whenever the tower is cleaned and disinfected (which should be done about twice a year). Where surface water from lakes, rivers, streams or reservoirs is used, antimicrobial treatment before the water enters the cooling system provides a practical and highly effective aid to control microbial fouling in the system.

It is often practical and highly effective to reduce the concentration of dissolved minerals, such as calcium and magnesium, in make-up water before it enters the cooling system (water softening). Water softening reduces the potential of the system to form biofilms, but may increase corrosion.

Reduction of organic load in the source water by chlorination or filtration (or both in concert) helps to remove nutrients that could lead to legionellae proliferation. Chlorination used to reduce the organic load may also serve to disinfect the water of its inherent microbial load.

Water treatment and water distribution — control measures

A system should be designed in such a way that water circulates through all parts of the system that should be wetted whenever it is operational. Deadlegs on existing systems should be removed or shortened (so that their length is no longer than the diameter of the pipe), or should be modified to permit the circulation of chemically treated water.

Dirt, organic matter and other debris should be kept to a minimum, as water treatment chemicals are generally more effective when the system is kept clean.

After stagnation of part or all of the system, system operation should always be coordinated with full chemical treatment of the water. Similarly, when a cooling tower system has been shut down for more than three days, the entire system (i.e. cooling tower, system piping, heat exchangers, etc.) should be drained to waste, if practicable. Since it is often not possible to completely eliminate all water from shut-down cooling systems, cooling water must be pretreated with an appropriate antimicrobial regimen before system start-up (HSC, 2000; Guideline 12–2000 in ASHRAE, 2000); that is, before activating the fans.

Corrosion inhibitors should be used to minimize corrosion of metal surfaces. Surfactants, biocides and other chemicals should be used to control fouling due to scale, silt and microbial growth. Use of these chemicals will help to maintain efficient heat transfer at metal surfaces, ensure free flow of water throughout the system and prevent the proliferation of microorganisms that are responsible for surface corrosion and degradation.

Disinfection — control measures

Because of the many factors that can compromise the disinfection process (outlined above), it is advisable to vary the antimicrobial stresses applied in the cooling water microbial control programme (McCoy, 1998), particularly in the case of non-oxidizing biocides. One practical and effective means to vary antimicrobial stresses is to alternate between two non-oxidizing biocides added as a single (“slug” or “shot”) dose, manually or automatically, at 3–4-day intervals. Another effective approach is to alternate use of an oxidizing antimicrobial with a non-oxidizing antimicrobial, to ensure that different modes of antimicrobial action are employed. When varying antimicrobial stresses, performance-based monitoring is used to assess the extent of microbial control achieved (McCoy, 2003).

The section on control measures for cleaning and maintenance (below) contains additional information on the disinfection process.

Oxidizing biocides

Commonly used oxidizing antimicrobials for cooling water include chlorine, bromine, stabilized bromine, combinations of bromine and chlorine, chlorine dioxide, peroxy compounds such as hydrogen peroxide and peracetic acid, and ozone (Kim et al., 2002; McCoy, 2002). Oxidizing antimicrobials are often effective when fed continuously using metering systems with small pumps, and many towers are successfully treated with continuous dosing with chlorine or bromine.

Shot-dosing of oxidants, which can also be very effective in microbial control, is an alternative to unvarying application of oxidizing antimicrobials.

Non-oxidizing biocides

Non-oxidizing biocides are most effective when shot dosed. The maintenance of a continuous residual of non-oxidizing biocides in the system will inevitably lead to the selection of resistant microorganisms and loss of microbial control (Russell, 2000; 2002). Non-oxidizing biocides are usually dosed at higher concentrations (15–50 parts per million [ppm]) than oxidizing biocides, and may require longer contact times at these concentrations (4–10 hours).

Treatment programme

All biocides should preferably be fed via a metering system, and the appropriate dose calculated on the basis of system volume and half-life (dilution rate) within the system (Kim et al., 2002).

“Blow-down” or “bleed-off” is the removal of some of the water periodically or continually, and its replacement with fresh water, to control the continuous accumulation of dissolved solids in the water. This process may be controlled by a conductivity controller that detects the increase in conductivity due to the dissolved solids, and automatically regulates the rate to hold a preset conductivity by triggering the operation of a solenoid drain valve.

Blow-down may be activated immediately before the addition of the biocide, to ensure that the amount of suspended dirt in the water that might react with and neutralize the biocide is minimized. Blow-down may then be stopped for a period after the addition of the biocide, to ensure that the chemical is retained at a sufficient concentration for long enough to be effective.

In selecting a chemical treatment programme, the operating parameters and water chemistry that may be unique to the system should be considered. A microbial control problem is rarely resolved by the application of generic technologies. Any microbial control strategy will fail without due attention to other control measures. Usually, the advice and the practical guidance of a water treatment specialist are necessary.

Where holding tanks are used, they can be disinfected by filling with water and chlorinating at 5 mg per litre free chlorine while maintaining the pH between 7.0 and 7.6. After one hour, this disinfected water can then be added to the cooling tower as part of the routine cleaning and disinfection procedure.

In emergency responses, systems must be cleaned, the water used for cleaning drained, and the system refilled. If the water used to refill the system is not clean and does not contain a disinfection residual, recontamination may occur, making it necessary to repeat the entire cleaning procedure.

Biofilms — control measures

To facilitate penetration of an antimicrobial into biofilms and sediments, use of a compatible and environmentally acceptable dispersant and/or detergent is strongly advised. There are many acceptable surfactant (surface-active) chemicals of this type, including non-ionic, anionic and amphoteric compounds (McCoy, 2003).

Temperature — control measures

Systems operating at the lower end of their working temperature range are likely to support less *Legionella* contamination (Kusnetsov et al., 1997). Therefore, systems should be designed to operate at the lowest possible temperature, to minimize legionellae growth (Bentham & Broadbent, 1993).

Design and materials used in construction — control measures

Cooling towers should be designed to:

- be easy to clean
- avoid the accumulation of sludge and deposits
- provide easy access for maintenance of internal surfaces, including the fill (Broadbent, 1996).

Connected tanks, filters and other devices must be scrutinized for their potential to support the proliferation of *Legionella*. Materials should be non-porous, with easy-to-clean surfaces, and should not provide nutrients for growth.

Cleaning and maintenance — control measures

A maintenance programme for the cooling system is essential, and the programme should be recognized and monitored as an important control measure.

The following procedures are effective for maintaining a clean system:

- regular physical cleaning, judicious use of chemicals and prevention of the build-up of dirt and dissolved solids in the circulating water

- periodic or continuous bleed-off or blow-down (excessive bleed-off should be avoided, because it will result in a loss or dilution of water treatment chemicals, and thus reduce the effectiveness of the treatment programme)
- addition of chemicals to the water at a rate sufficient only to maintain predetermined chemical concentrations and a stable total bacteria count below a predetermined acceptable level
- regular checks of tower components
- cleaning of wetted surfaces
- water treatment to minimize corrosion and scaling, and to provide biological control
- routine cleaning and disinfection
- regular visual inspections for general cleanliness
- cleaning of the sump of the unit when any build-up of dirt, organic matter or other debris is visible or found through sampling (effective means of removing particulate matter include side-stream “filtration”, coupled with strainers, cartridge filters, sand filters, centrifugal-gravity-type separators or bag-type filters).

The aim of the maintenance programme is to ensure optimal thermal performance and also to minimise the risk of disease, through a combination of mechanical maintenance and total tower cleanliness. The programme should cover regular water treatment, inspections and cleaning, and should be implemented as soon as the cooling tower starts to operate. Box 5.3 provides an example of a corrective action procedure for emergency disinfection. The type of situation that might lead to corrective action is finding that monitoring results suggest the tower is out of control; for example, if results show a failure of biocide dosing, or repeated high counts of total viable bacteria or legionellae.

Box 5.3 Example of corrective action procedure for emergency disinfection and cleaning

If a cooling tower water system has been implicated in an outbreak of Legionnaires' disease, emergency disinfection and cleaning of that system must take place as soon as possible. The following actions should be taken where appropriate:

- switch off the fan immediately
- take samples for laboratory investigation before any further action
- switch off the circulation pump as soon as practicable and decommission the system
- consult the enforcing authority before proceeding further
- keep all personnel clear of the tower area
- when the area has been cleared by the enforcing authority, add sodium hypochlorite to the system water to obtain a measured concentration of 50 mg/l of free chlorine, and add a suitable biodispersant to prevent biofilm formation
- where possible, cover the air inlet and outlets with plastic sheeting or similar material during disinfection, to prevent the release of aerosols from the tower (partially damaged biofilm may be sloughed off, homogenised by the pumps and aerosolised by the water distribution system, potentially creating a risk until the disinfectant has had time to be effective)
- where possible, check the pH and, if it is >8.0, reduce it
- circulate the system water with the fans off for a period of at least 6 hours
- maintain the free chlorine level at an absolute minimum of 20 mg/l at all times
- after six hours, de-chlorinate and drain the system
- undertake manual cleaning of the tower, sump and distribution system, with cleaning staff wearing full pressurised respirators
- refill with fresh water and add sodium hypochlorite
- recirculate without using the fan, at 20 mg/l of free chlorine for six hours
- de-chlorinate and drain the system
- refill, recirculate and take samples for testing
- re-commission the system when test results detect no legionellae and/or permission is granted by the enforcing authority.

Source: Adapted from HSE 2004

The procedure detailed in Box 5.3 for emergency disinfection is, in most circumstances, also appropriate for general disinfection and cleaning. However, there may be site- or industry-specific procedures that should be used, and local environmental health authority regulations that must be observed. Copies of the applicable procedure, and records of all actions and any test results, must be maintained on site at all times. Disinfection using chlorine, bromine, chlorine dioxide or another approved antimicrobial must be in accordance with local legislation. Particular points to be noted when cleaning and disinfecting are shown in Box 5.4.

Box 5.4 Points to be noted when cleaning and disinfecting

When cleaning and disinfecting a cooling tower or evaporative condenser, it is important to:

- minimize creation of aerosols
- where possible, remove drift eliminators, inspect fill and packing, and clean and repair or replace as required
- clean all water filters and strainers associated with the distribution system
- check water distribution nozzles or troughs, and gutters, and clean or replace as required
- use a low-pressure spray of a combination detergent and oxidizer (e.g. sodium hypochlorite) for cleaning; do not use high-pressure washers on plastic packing or eliminators (if high-pressure washers are to be used on other parts of the tower, the washing should be covered, to contain most of the splashing)
- manually clean the tower, packing, sump, eliminators and distribution system; ensure that the cleaning is timed to minimize the risk of exposing individuals in the vicinity of the tower, and that personnel wear positive-pressure high efficiency particulate absorbing (HEPA) filter masks (respirators) during the procedure
- remove high-density plastic film pack for cleaning, if recommended by relevant authorities — some authorities recommend this, because it can be difficult to check after cleaning that the interstices in the pack are free of dirt and scale (HSC, 2000), but there are no published studies comparing the effectiveness of cleaning in place with removing the pack for cleaning
- ensure that records of the procedures are kept, and they include the date and the signature of the responsible party or authority.

Spray drift — control measures

The effectiveness of drift eliminators varies, depending on their design and condition —state-of-the-art eliminators are significantly more efficient than older designs. The eliminators should be inspected regularly (at least every six months) and either cleaned and disinfected or replaced, as necessary. Shorter intervals between inspection and cleaning may be advisable for systems in which heavy fouling is a chronic occurrence, or where highly susceptible populations are likely to be exposed (HSC, 2000).

5.4.2 Monitor control measures

This step involves defining the limits of acceptable performance and how these are monitored. The results of tests such as those listed above allow corrective actions (discussed in Section 5.5.3, below) to protect public health to be taken.

Legionella populations in cooling systems are highly variable, and elevated concentrations occur sporadically in most cooling towers (Bentham, 2000), meaning that single measurements show only a snapshot of the microbial situation. Since it is not possible to monitor *Legionella* concentrations continuously, other strategies must be used to maintain concentrations as consistently low as possible; one such strategy is to prevent situations that stimulate growth of the bacteria.

Regular monitoring of *Legionella* concentrations should be included to build up a picture of the trend. *Legionella* tests are not recommended as a guide for control measures, because their inherent unreliability means that the results cannot be used as a reproducible, sensitive and timely measure of system control (see Chapter 11). *Legionella* testing should only be used to verify and validate a WSP — test results should not be seen as a surrogate for a comprehensive control strategy (Bentham, 2002).

Conditions and frequency of testing

The heterotrophic plate count (HPC) technique (also known as heterotrophic colony count, total colony count, total viable count and total heterotrophic count) is useful in assessing the efficacy of antimicrobial treatments of cooling tower water (WHO, 2004).

Microbial testing should preferably be carried out in a laboratory that is competent and accredited to do this work. If dipslides are used to test environmental samples (e.g. from cooling tower waters), an incubator should be used for temperature control, and the slides should be incubated at 30 °C for at least 48 hours before interpretation of the result. Dipslides are simple, convenient and inexpensive, but their accuracy is limited. They are useful in detecting major changes in bacterial levels and for verifying that a water treatment programme is being implemented. Periodic counts by the agar plate method are required for a more reliable and reproducible assessment of HPC. Regular (e.g. monthly) HPC on tower water should be undertaken, to assess the efficacy of the biocide treatment and general cleanliness of the system. A count of 5×10^5 colony forming units (CFU)/ml in HPC is an acceptable upper limit for treated tower water in a clean system (HSE, 2004). If this level of HPC is exceeded, the frequency of testing should be increased to weekly, until control has been re-established.

Culture techniques and detection limits

Deficiencies in culture sensitivity and precision (discussed in Chapter 11) diminish the use of action levels as a meaningful control measure in cooling water systems. Any *Legionella* test result should be considered in the light of the detection limit of the method used, which should be clearly reported with test results.

Sampling

Chapter 11 discusses requirements for sampling.

Where open basins are involved, water samples should be taken below the surface of the water. When samples are obtained from taps, it is preferable to select those that are connected directly to pipes containing the circulating water. Sample taps should be clean and free of leaks and external fittings, such as hoses. Taps should be run so that the entire length of the fitting is flushed with water for at least 30 seconds before taking the sample.

In certain circumstances, samples may be taken from locations that are not representative of the bulk of the tower water. It may often be of interest to include sediment in the sample to be analysed. When investigating a cooling tower implicated as a potential source of legionellosis infection, it is essential to collect swab samples of biofilm in the sump or basin of the tower.

Timing of sampling for bacteriological analysis is important, particularly when shot dosing of biocides is used. Sampling is best undertaken just before shot dosing, as this will demonstrate the “worst case scenario” in the dosing cycle, and may indicate the need for corrective action in the water treatment or cleaning programme.

Analytical results should be recorded as part of the documentation in the WSP. Records should be kept for a period in accordance with local or national legal requirements.

Non-microbial tests

In addition to microbial testing, a number of other tests can be carried out on site, using samples of cooling tower water. Most analyses are for parameters related to control of corrosion, scale and particulate matter; they include measurements of temperature, pH, conductivity, chloride, alkalinity, chromate and organophosphonate. Analysis of antimicrobials is generally limited to oxidizing chemicals such as chlorine, bromine or chlorine dioxide. The concentrations of non-oxidizing biocide can be determined in a well-equipped chemical laboratory, although the methods used are often time consuming and expensive. Turbidity may be determined in a laboratory or on site with a portable turbidity meter.

5.5 Management and communication

This section should be read in conjunction with Section 3.3.3 of Chapter 3. The steps involved in management and communication are to:

- develop supporting programs (Section 5.5.1)
- prepare management procedures (Section 5.5.2)
- establish documentation and communication procedures (Section 5.5.3).

5.5.1 Develop supporting programs

A supporting programme should be established, to train all operating staff to perform the required monitoring and maintenance tasks. Also essential is authorization of regular servicing and data collection by the owner of the building where the tower or condenser is located, or their nominated representative.

5.5.2 Prepare management procedures

This step involves preparing management procedures, including corrective actions, for normal and incident conditions. Corrective actions include repairing defects, and possibly re-treating or discarding water that might be contaminated, to ensure that unsafe water is not supplied.

For most cooling tower systems, if HPC results exceed 5×10^5 CFU/ml or show a dramatically increasing trend, an investigation should be carried out to determine the cause. The investigation should include:

- a check of the chemical dosing — where manual slug dosing of antimicrobial is undertaken, an examination of the relevant inspection reports concerning the amount and frequency of antimicrobial addition; where an automatic dosing system is installed, the check includes:
 - a check of the level of biocide in the storage container
 - an examination of the metering system for leaks, blockages and correct delivery of antimicrobial into the cooling tower water
- internal and external examination of the cooling tower should be for cleanliness, deterioration of construction materials and signs of tower pollution, including checking of the bleed-off rate.

Based on the results of investigations, corrective action may include:

- a complete review of the water treatment programme, in cooperation with a water treatment specialist
- remedial action, as required
- implementation of a routine cooling tower cleaning and disinfection procedure
- repeat water sampling for HPC after corrective procedures have been implemented
- performance of a new risk assessment after all corrective measures have been implemented.

5.5.3 Establish documentation and communication procedures

Table 5.2 gives an example of documentation for a routine monitoring and corrective action loop.

Table 5.2 Example documentation for monitoring and corrective action

Process step	Indicator	Monitoring		Operational limit	Corrective action	
Chlorination	Free chlorine	What	Chlorine	Not less than 0.5 ppm	What	Check pH of water and the chlorine dosing pump
		How	Diethyl-p-phenylene diamine test kit		How	pH probe and observation of the pump
		When	Weekly		When	Immediately
		Where	Tower basin		Who	Building engineer

ppm = parts per million

If an outbreak of legionellosis occurs in the vicinity of a tower, that tower should be considered as a potential source of infection; a precautionary decontamination is appropriate. Water samples should be taken from the most contaminated point, and tests commenced immediately. The standard from the International Organization for Standardization ISO 5667 (ISO, 2001) can be used in developing sampling methods. Samples should be taken as near to the heat source as possible. The suspected tower can then be immediately decontaminated, rather than waiting for the results of the bacteriological tests, which may take 3–14 days.

In an outbreak, an emergency corrective action will be required, and procedures should already be in place and staff trained in their implementation (e.g. see Box 5.3, above).

5.5.4 Verification

As part of a regular auditing programme, the operator or their water treatment company should inspect cooling towers at least monthly (although shorter intervals may be determined by the risk assessment for the system). Additional access hatches may need to be provided, to facilitate inspection and cleaning of parts of the tower.

In a system in which risk assessment indicates cause for concern, verification of control of the system may be established by the routine assessment of *Legionella* concentrations in the cooling system. This verification should not precede or replace the routine monitoring of control measures established for the system.

Isolation of *Legionella* from any system is likely to occur occasionally (Bentham, 2000). Conducting comprehensive system risk assessments after positive test results should reduce the incidence of such results. Periodic review of the number of positives recorded should be used to assess whether a consistent level of control has been achieved and whether overall control is improving. After the introduction of a new WSP or significant modification to the system, the microbial test parameters described above should be applied. Verification of the other control measures determined for the WSP should precede microbial testing.

With the fan off, the water flow throughout the tower should be viewed for unrestricted flow from inside. If possible, drift eliminators should be examined for damage or excessive drift, from both inside and outside.

The condition of the internal structure of the tower should be examined, with the fan, the water pump and any dosing and filtering equipment switched off. Any deterioration of materials (wood, metal, etc.) should be noted, particularly of the fill, the drift eliminator, the basin and the water distribution system; a check should also be made for visible microbial growth.

More detailed inspections should be undertaken if the plant is shut down completely during annual inspection. This provides the opportunity for examining the interior of pumps, sections of pipework and heat exchange equipment.

5.6 Surveillance

The system assessment should be independently reviewed periodically (e.g. every two years) and after any major changes to the system or management. The review should be undertaken by a formal, competent authority.

The tower should be inspected under normal working conditions by an independent surveillance team, wearing appropriate safety equipment to prevent the inhalation of aerosols. A number of items can be examined externally; for example, the team might look for signs of microbial growth, algae, water leaks, splashing and blockages or restrictions at air inlets. Where chemical dosing equipment is installed, it should be examined for correct operation and for adequate stock of chemicals.

Details of maintenance should be recorded to identify performance trends and for prompt attention to faults reported by operational staff or building occupants. Maintenance procedures should be constantly monitored to ensure adherence to clearly defined objectives. Any changes to plant operation or modifications should be recorded in the maintenance manual.

Chapter 6 Health-care facilities

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This chapter describes how a water safety plan (WSP) can be applied to assessing and managing the risks associated with *Legionella* in health-care facilities. Infections acquired in a health-care setting are referred to as “nosocomial”.

This chapter looks at different infection reservoirs in hospitals, such as cold and hot-water systems or plumbing systems, cooling towers, bathing pools and dental units. For information on infection control measures in cooling towers and bathing pools, the reader should consult Chapters 5 and 8, respectively.

This chapter should also be read in conjunction with Chapter 3, which discusses the different elements that make up a WSP, and shows how a WSP fits within the framework for safe water quality developed by the World Health Organization (WHO). As explained in Chapter 3, a WSP has 10 steps that fit within the three main areas of system assessment, monitoring and management and communications (see Figure 3.2). A WSP must be comprehensive, and all 10 steps should be implemented in assessing and managing the risks associated with *Legionella*. However, this chapter focuses on parts of the WSP where information specific to health-care facilities is needed.

6.1 Background

In this chapter there is a risk assessment concerning different infection reservoirs in hospitals, such as cold and hot-water systems or plumbing systems, cooling towers, bathing pools and dental units. For infection control measures focusing on cooling towers and bathing pools, refer to the relevant chapter of this guideline.

A WSP needs to be comprehensive; however, an overview of such a plan is shown in Table 6.1, as an example of the type of information a plan might contain. As explained in Chapter 3, a WSP is part of a framework for safe water quality that also includes health-based targets and surveillance.

Nosocomial cases usually make up a small proportion of reported cases of legionellosis. However, the proportion of cases that are fatal tends to be much higher with nosocomial infections than with community-acquired infections. Therefore, health-care facilities have a special responsibility for preventing Legionnaires’ disease.

Health-care facilities include hospitals, health centres, hospices, residential care facilities and dialysis units. These institutions are settings in which people with predisposing risk factors for *Legionella* infections are more likely to be present, and in which medical devices that can

disseminate *Legionella* into the lower respiratory tract are used (such as medical humidifiers, inhalation devices and respiratory therapy equipment). Retirement homes should be considered with health-care facilities, as people with predisposing risk factors are likely to live there; and several cases of legionellosis have been reported among residents of retirement homes (Campese & Declut, 2002b).

Cooling towers were originally thought to be the main source of nosocomial legionellosis, after the bacteria were isolated from a cooling tower near a hospital dealing with cases of Legionnaires' disease (Dondero et al., 1980). For example, the world's biggest outbreak of legionellosis (Murcia, Spain in 2001 with 449 confirmed cases) was shown by epidemiological and microbiological investigation to be associated with the air-conditioning cooling towers of a city hospital (Garcia-Fulgueiras et al., 2002). However, many nosocomial cases have been associated with piped hot and cold-water distribution systems (Sabrià & Yu, 2002); ice made with water containing legionellae has also been incriminated as a source of infection in hospitals, when patients have been given ice cubes to suck (Stout, Yu & Muraca, 1985).

Underlying disease is a major risk factor for acquiring Legionnaires' disease. Since the major mode of transmission is aspiration, patients with chronic lung disease or those who undergo surgery requiring general anaesthesia are at greater risk. One of the highest incidence rates of nosocomial Legionnaires' disease was in a population of surgical head and neck cancer patients. This group of people has a propensity for aspiration, as a result of their oral surgery (Johnson et al., 1985). Nasogastric tubes have been linked to nosocomial legionellosis in several studies, with microaspiration of contaminated water the presumed mode of entry (Blatt et al., 1994; Venezia et al., 1994). It is unlikely that colonization of the oropharynx by *L. pneumophila* leads to transmission (Bridge & Edelstein, 1983; Pedro-Botet et al., 2002).

Heart transplant patients have been shown to have a high incidence of Legionnaires' disease (Hofflin et al., 1987; Mathys et al., 1999), whereas bone marrow transplant patients have a low incidence (Chow & Yu, 1998). Corticosteroid administration is an independent risk factor (Carratala et al., 1994; Lepine et al., 1998).

For people with predisposing risk factors, there is not only a higher risk of infection but also a higher case-fatality rate (up to 50%) than in other settings, as a consequence of their often immunosuppressed or predisposing status (Yu, 2000). Paradoxically, patients with acquired immunodeficiency syndrome (AIDS) appear not to be at increased risk for nosocomial Legionnaires' disease (Gutiérrez et al., 1995).

6.1.1 Surveillance data on nosocomial Legionnaires' disease

Between 1980 and 2001, 4021 cases of Legionnaires' disease in residents of England and Wales, United Kingdom were reported to the Communicable Disease Surveillance Centre's National Surveillance Scheme — an average of 183 cases per year. Of the total number of cases, 269 were linked to hospital-acquired infection (PHLS, 2002).

For 1999 and 2000, a total of 384 cases of Legionnaires' disease among residents of England and Wales were reported to the Public Health Laboratory Service. Of these patients, 19 (5%) acquired their infection in hospital. In 1999, there were seven single cases of Legionnaires' disease among residents of England and Wales. Five of the seven people affected were immunosuppressed, two were renal transplant patients and one was a cardiac transplant patient. In 2000, 12 cases with five deaths were considered to be nosocomial. Half of these cases were immunosuppressed, and three were associated with an outbreak in a hospital that had had a previous outbreak (PHLS, 2002).

Table 1.7 in Chapter 1 shows the exposure setting for Legionnaires' disease cases in France between 1999 and 2002. Due to an improvement in notification of legionellosis, the total annual number of cases reported in France from 2000 to 2003 increased. However, the percentage of nosocomial cases decreased annually and significantly, from 20% in 2000 to 9% in 2003. During the same period, the percentage of cases reported in people staying in hotels and at camp sites increased from 9% to 13% (Campese, 2004). This has been interpreted as a reflection of the impact of measures taken by health institutions to control the risk of Legionnaires' disease following a ministerial circular in 1998.

6.2 Water safety plan overview

A WSP needs to be comprehensive; however, an overview of such a plan is shown in Table 6.1, as an example of the type of information a plan might contain. As explained in Chapter 3, a WSP is part of a framework for safe water quality that also includes health-based targets and surveillance.

The remainder of this chapter provides information relevant to a WSP specific for potable water and in-building distribution systems, for each of the three main areas of a WSP:

- system assessment (Section 6.3)
- monitoring (Section 6.4)
- communication and management (Section 6.5).

Sections 6.3–6.5 should be read in conjunction with Section 3.3 from Chapter 3.

Fundamentally, the responsibility for managing the risk of legionellosis belongs to the owner or manager responsible for the potable water or in-building distribution system. To ensure that the WSP is properly implemented, the owner or manager of the facility should assign tasks, ensure that documentation is complete and current, and hold people accountable.

Table 6.1 Water safety plan overview

Process step	Water source	Distribution	Respiratory apparatus
Assess hazards and prioritize risks (example) ↓	High nutrients and microbial load in source water	Stagnant water in deadlegs in the pipework, resulting in proliferation of legionellae	Legionellae entering respiratory apparatus in tap water, being inhaled by patient and leading to potential Legionnaires' disease
Identify control measures (example) ↓	Routine disinfection of water at 0.3–0.5 mg/l free residual chlorine (depending on the national regulations)	Routine cleaning procedures for distribution system, and review of system flow diagram	Use of sterilized or point-of-use filtered water to clean respiratory equipment Cleaning and disinfection protocol for respiratory apparatus; microbiological monitoring programme
Monitor control measures (example) ↓	Chlorine on line with chlorine/redox probe Turbidity on line	Chloramination ^a Routine review of process flow diagram (desktop and onsite) to identify areas of concern or stagnation	Monitoring of water sterilization devices Monitoring of cleaning protocol records
Prepare management procedures (example)	Point-of-use filtration and disinfection programme; possible treatment for dissolved solids	Removal of deadlegs where possible	Isolation of unit and disinfection of source
Establish verification and surveillance (example)	• Internal audit and external audit (by the health department) to confirm that operational monitoring and corrective actions are being undertaken as stated in the WSP • Monthly heterotrophic colony counts at the tap and in the source water (to track trends and changes, rather than as an absolute indicator, and to be undertaken by an accredited laboratory) • Three-monthly sampling for legionellae of water in the distribution system and at the point of use • Respiratory apparatus must be disinfected on a regular daily basis and between every patient; also, it must be regulated in the hospital hygiene plan		
Develop supporting programmes (example)	• Staff training and education; maintenance and calibration		

^a Not currently available for individual buildings. In Europe the levels permitted are lower than in USA which may affect the results.

6.3 System assessment

This section should be read in conjunction with Section 3.3.1 of Chapter 3. The steps involved in system assessment, some of which are discussed further below, are to:

- assemble a team to prepare the WSP
- document and describe the system (Section 6.3.1)
- assess hazards and prioritize risks (Section 6.3.2)
- assess the system.

6.3.1 Document and describe the system

A system assessment for health-care facilities should consider the well-described infection reservoirs in community-acquired Legionnaires' disease; for example, potable and in-building water systems (discussed in Chapter 4) and cooling towers and evaporative condensers (discussed in Chapter 5); in addition, the assessment should assess the type of health care provided, and the immune and health status of the individuals using the facilities. Table 6.2 details the types of system components that should be considered.

Table 6.2 Examples of system components to be considered in system assessment and subsequent hazard analysis in health-care facilities

System component	Comment	Reference
Hot and cold-water systems	Evidenced and epidemiologically based associations	See Chapter 4
Cooling towers and evaporative condensers		See Chapter 5
Respiratory devices (including nebulizers and ventilatory machines)		Levy & Rubin (1998)
Medical humidifiers filled and rinsed with tap water		Levy & Rubin (1998)
Birthing pool water		Levy & Rubin (1998)
Drinking water dispensers (not discussed further in this chapter)	Any epidemiological links are unclear. An investigation of drinking water dispensers in hospitals found <i>Legionella</i> in 4 out of 50 dispensers. An association with <i>Legionella</i> infections was not investigated.	Rechenburg, Engelhart & Exner (2001)
Water systems in dental units (not discussed further in this chapter)	These have sometimes been shown to be heavily colonized with <i>Legionella</i> , particularly where there are multiple chairs (e.g. in dental schools), but no cases of Legionnaires' disease have been attributed to dental units. Dentists have been found to have high titres of <i>Legionella</i> antibodies.	Pankhurst et al. (1990, 2003)

6.3.2 Assess hazards and prioritize risks

This step involves collecting and evaluating information on specific hazards associated with health-care settings, and conditions leading to their presence, to decide which are significant for safety and therefore should be addressed in a safety plan. The hazards considered here include those associated with the system components listed in Table 6.2.

Hot and cold-water systems — risk factors

The risk of nosocomial Legionnaires' disease associated with the colonization of hot and cold-water systems by *Legionella* is well established. For example, Joly & Alary (1994) performed a follow-up study for 9 months at 20 hospitals, and found that the 10 hospitals containing readily detectable *Legionella* experienced significantly more frequent cases of Legionnaires' disease than did the 10 hospitals where *Legionella* was not readily detected ($P = 0.054$).

A five-year prospective study in 20 hospitals in Spain analysed the incidence of new cases of nosocomial legionellosis. In 64.7% of hospitals, *Legionella*-positive water cultures were found and nosocomial legionellosis was diagnosed; however, in hospitals where *Legionella* was not detected, no nosocomial legionellosis was reported. The reported incidence of nosocomial legionellosis has increased significantly since environmental studies increased detection of the organism (Sabrià et al., 2004).

The proportion of distal sites in the water system of a hospital that are positive for *Legionella* directly correlates with the incidence of Legionnaires' disease; that is, the greater the percentage of sites holding *Legionella*, the more likely it is that cases will occur. The opposite is also true — if *Legionella* is not detected in the water supply, cases will not occur (Stout & Yu, 2001).

Based on the evidence of a link between the colonization of hot and cold-water systems in hospitals and other buildings and the risk of a *Legionella* infection, Exner et al. (1993) investigated hospitals, residential units and other buildings that could be affected by the colonization of water systems with *Legionella*. The study distinguished between:

- a local or non-systemic colonization (defined as a colonization of isolated parts of the system, such as water outlets or shower heads)
- a systemic colonization of the water system (defined as a colonization of the whole system, including the central parts of the water supply).

Table 6.3 shows the type of colonization of water distribution systems by *Legionella* in health-care facilities in Germany.

Table 6.3 Type of colonization of water distribution systems by *Legionella* in health-care facilities in Germany

Building type	Type of colonization		
	Systemic number	Local number	Not detected in 1 litre
Hospitals (<i>n</i> = 73)	46 (63%)	11 (15%)	16 (22%)
Residential institutions (<i>n</i> = 77)	28 (36%)	9 (18%)	40 (52%)
	Concentration range		
	>10 000/l	100–10 000/l	<100/l
Building type	Type of colonization		
	Systemic number	Local number	Not detected in 1 litre
Hospital (<i>n</i> = 46)	23 (50%)	17 (37%)	6 (13%)
Residential institutions (<i>n</i> = 28)	17 (61%)	8 (29%)	3 (11%)

Source: Adapted from Exner et al. (1993)

In a study in Spain, *L. pneumophila* was isolated from 17 out of 20 (85%) hospital potable hot-water systems (Sabrià et al., 2001). Each hospital had its own unique DNA (deoxyribonucleic acid) subtype, reflecting systemic colonization (as defined by Exner et al., 1993, above).

Cooling towers and evaporative condensers — risk factors

Because evaporative condensers are an important potential infection source for hospital-acquired pneumonia, they are considered briefly here, although they are covered in detail in Chapter 5.

In Germany, investigations of evaporative condensers mainly in hospital areas found high concentrations of *L. pneumophila*, with 13 out of 15 condensers having concentrations above 10⁴ CFU/litre (colony forming units per litre) (Pleischl, Krizek & Exner, 2002). The causes of the high concentrations were insufficient cleaning and disinfection, and low maintenance of the evaporative condensers.

In hospitals, the risk of legionellosis from cooling towers appears to be much higher than the risk from showers. According to this risk assessment model (Ambroise & Hartemann, 2005), the annual median risk of clinical legionellosis cases for people exposed to daily showers is (Prof P Hartemann, Faculté de médecine de Nancy, pers comm, July 2006):

- less than 1 in 100,000 people for a concentration of less than 1000 CFU/litre of *L. pneumophila* serogroup 1 in hot water
- less than 1 in 10,000 people for concentrations of more than 2 × 10⁵ CFU/litre of *L. pneumophila* serogroup 1 in hot water.

A risk assessment for cooling towers and evaporative condensers in health-care facilities should take into account the proximity of cooling tower exhausts to the air inlets for wards housing high-risk patients, such as those who have undergone renal transplants.

Respiratory apparatus and tubing — risk factors

In addition to the normal inhalation risks, patients in health-care facilities are at greater risk when forced to inhale water in respiratory devices that may contain legionellae (Marrie et al., 1991; Blatt, Parkinson & Pace, 1993; Yu, 1993; Venezia et al., 1994; Kool et al., 1998). For example, inhalation of contaminated aerosols may occur when tap water is used to rinse or fill respiratory devices, tubing for use in mechanical ventilation machines and chambers of hand-held medication nebulizers. Nosocomial aspiration pneumonia has been reported in patients, particularly after surgery where there is intubation (Blatt, Parkinson & Pace, 1993; Yu, 1993; Venezia et al., 1994). Patients with Legionnaires' disease were found to have undergone tracheal tube placement significantly more often or to have been intubated for significantly longer than patients with other types of pneumonia (Yu, 2000).

A retrospective review of microbial and serological data from the laboratories of a hospital in the United States of America (USA) dealt with clusters of cases of Legionnaires' disease among hospitalized patients (Kool et al., 1998). By reviewing the charts of patients over a period of 10 years, the authors identified 25 culture-confirmed cases of nosocomial or possibly nosocomial Legionnaires' disease, in which 12 patients (48%) died. For cases that occurred before 1996, intubation was associated with increased risk of disease. High-dose corticosteroid medication was strongly associated with a risk for disease. Six or seven available clinical isolates were identical and were indistinguishable by pulse-field gel electrophoresis from environmental isolates from the water system.

Birthing pool water — risk factors

The important role of pool water — especially of hot tubs — as infection reservoirs of *Legionella* is well established (see Chapter 8). The first report of a newborn contracting *L. pneumophila* pneumonia after water birth was in 2001 (Franzin et al., 2001). Because the hospital water supply and, particularly, the pool water for water birthing were contaminated by *L. pneumophila* serogroup 1, the newborn was infected — perhaps by aspiration — after a prolonged delivery in the contaminated water.

6.4 Monitoring

This section should be read in conjunction with Section 3.3.2 of Chapter 3. The steps involved in monitoring, some of which are discussed below, are to:

- identify control measures (Section 6.4.1)
- monitor control measures (Section 6.4.2)
- validate effectiveness of the WSP.

6.4.1 Identify control measures

This section should be read in conjunction with Chapters 4 and 5, which provide control measures for potable water, in-building systems, cooling towers and evaporative condensers. Control measures must be implemented in evaporative condensers installed in or near hospitals. In Britain, most cooling towers have been removed from hospitals following a major outbreak of legionellosis in 1985 (J Lee, Health Protection Agency, UK personal communication, June 2005). Where high-risk patients are housed, additional precautions should be considered, such as installation of high-efficiency particulate absorbing (HEPA) filters on the air inlet and monitoring of both the cooling systems and patients. One of the most effective control measures is to maintain a temperature outside the range of 25–50 °C in the network, as discussed in Chapter 3, Section 3.3.2.

Hot and cold-water systems — control measures

The guidance given here relates to general hospital hot and cold-water systems. In high-risk areas, such as transplant centres and intensive care units, water from the outlet should be free of *Legionella* (no colonies detectable in 1 litre of water). If this cannot be achieved within the system then point-of-use filters will be needed at the outlet. Ice should be made either from water that has had *Legionella* removed by filtration, or from heat-sterilized water.

If there is only an isolated colonization of a distal site, it is possible to flush out *Legionella* from the site (for example, from a water tap). In the case of a systemic colonization of the water distribution system, even intensive flushing causes no sustained reduction of legionellae.

In one study mentioned above (Kool et al., 1998), the water system was extensively modified, and no further cases were identified in the hospital in the following year. The authors concluded that *Legionella* can colonize hospital potable water systems for long periods, resulting in an ongoing risk for patients, especially those who are immunocompromised. In the investigated hospital, nosocomial transmission possibly occurred for more than 17 years before it was finally interrupted in 1996 by extensively modifying the water system as a substantive control measure.

Point-of-use filters may also be used to mitigate the risk of legionellae.

Analysis of hot and cold-water systems for *Legionella* is no substitute for control measures; rather, it is a verification that control measures are working.

Respiratory apparatus and tubing — control measures

Water that is used to rinse and clean respiratory apparatus should be sterile.

Because of the seriousness of nosocomial *Legionella* infections and the availability of low-cost sterile water (proven to be effective in reducing proliferation of legionellae), sterile water should be used in high-risk equipment such as respiratory devices, to avoid exposing at-risk hospitalized patients to hospital water. Sterile water should also be used for rinsing and cleaning humidifiers, nebulizers and respiratory machines.

Birthing pool water — control measures

Birthing pools should be designed for the purpose, and should be physically cleaned and disinfected both before and after birth (noting that the high amount of organic material will inactivate residual biocides). Where hoses are used for filling, they and any connectors should be disinfected before use. A risk assessment and pool management plan should be designed that takes into account the intermittent use and storage conditions of the pool. Disposable liners are available for pools.

Disinfection — control measures

Monochloramine is likely to be more effective for disinfection than free chlorine, because it is more resistant and a residual is more likely to persist to the point of delivery; also, it is more likely to penetrate biofilms. Hospitals supplied with drinking-water treated with monochloramine as the residual disinfectant have been shown to be less likely to have a reported outbreak of Legionnaires' disease than those using water treated with free chlorine. In contaminated hospitals, the proportion of sites testing positive was inversely related to the free residual chlorine concentration ($P = 0.01$) (Kool, Carpenter & Fields, 1999; Kool et al., 1999).

6.4.2 Monitor control measures

This step involves defining the limits of acceptable performance and how these are monitored (i.e. what will be monitored, and how, when and by whom). Again, this section should be read in conjunction with Chapters 4 and 5.

To protect the most vulnerable patients in hospitals, there are distinct requirements, which depend on the risk estimation. For example, one requirement might be to maintain legionellae-free water in systems that produce aerosols in showers, in wards or in hospital rooms where there are immunocompromised patients.

The results of monitoring allow corrective actions (discussed in Section 6.5.1, below) to protect public health to be taken.

6.5 Management and communication

This section should be read in conjunction with Section 3.3.3 of Chapter 3. The steps involved in management and communication are to:

- develop supporting programs
- prepare management procedures (Section 6.5.1)
- establish documentation and communication procedures (Section 6.5.2).

6.5.1 Prepare management procedures

This step involves preparing management procedures, including corrective actions, for normal and incident conditions. Box 6.1 provides an example of limit values set for *Legionella* concentrations in water used in health-care settings.

Box 6.1 Example of limit values for *Legionella* concentrations and microbiological indicators in water used in health-care settings in France

Limit values

For patients with classical individual risk factors such as the elderly, those with alcoholism or tobacco addiction:

- target level <1000 CFU/l *Legionella pneumophila*
- alert level 1000 CFU/l *Legionella pneumophila*
- maximum level 10,000 CFU/l *Legionella pneumophila*

For high-risk patients, such as those with severe immunodepression, transplantation, corticotherapy with an equivalent dose of 0.5 mg/kg per day prednisolone for 30 days or more, or 5 mg/kg per day for 5 days or more:

- target level not detectable
- alert level 250 CFU/l *Legionella* spp.

Microbiological indicators

Aerobic flora at 22 °C and 36 °C. No variation above a 10-fold increase compared with the usual value at the entry point. One control per 100 beds per year, with a minimum of four controls per year.

- *Pseudomonas aeruginosa* <1 CFU/100 ml quarterly
- total coliforms <1 CFU/100 ml quarterly

Values may vary in other countries. Control measures should be implemented, these could include “point-of-use filters” fitted at the outlets.

Because no detailed risk assessment has focused on the immunosuppressed, these values are based on the precautionary principle.

Source: Adapted from Ministère de la Santé et des Solidarités (2005)

Samples must be taken immediately:

- if there are signs that the water system is not under control
- after periods of stagnation
- after work on the distribution system, etc.

The target levels defined in Box 6.1 are seen as the best way to minimize the risk. The alert level is designed to ensure that relevant people are informed, and that corrective actions (e.g. a review of the procedure for maintenance, or new controls) are instigated. When the maximum level is reached, disinfection of the water distribution system must be organized and the procedure for maintenance revised. Additionally, an independent body (e.g. local sanitary authorities) should carry out a new inspection before authorization for reuse is given.

Operationally, control measures, such as temperature, disinfectant residual and pH should be monitored on line, as discussed in Chapters 4 and 5.

Every case of nosocomial legionellosis constitutes an alert, meaning that other cases may have occurred or could occur in the future in the health-care facility (which would constitute an outbreak; see Box 6.2). Where there is a possibility of a nosocomial case, it should always be investigated.

Box 6.2 Definition of a nosocomial outbreak

A nosocomial outbreak is defined as two or more confirmed cases of legionellosis in the same hospital or residential institution within a six-month period.

Location of the outbreak is defined in terms of geographical proximity of the cases and requires a certain level of judgement.

The WSP and system assessment for control of *Legionella* in the hospital and the maintenance records must be reviewed by the following people, working together:

- the incident and outbreak management control team
- the person responsible for *Legionella* control
- the appropriate hospital engineer
- the infection control physician (hospital hygienist).

The aim of the review is to ensure that the preventive procedures identified as necessary to prevent proliferation of and exposure to *Legionella* are followed. Any deficiency in the control procedures should be remedied as soon as possible. Sampling should be undertaken, followed by precautionary disinfection of parts of the water system, if this is considered to be justified.

The incident and outbreak control management team should always include an expert in environmental monitoring of *Legionella*. Researchers should not be confined to the index case; it is important to also look for other previously undetected cases of legionellosis. The search should look for other confirmed or presumptive cases of Legionnaires' disease associated with the hospital or community, unexplained cases of nosocomial pneumonia in patients (especially those with impaired immunity), and pneumonia in hospital staff.

Until the situation is under control, a critical review of every presumptive diagnosis of pneumonia in the hospital or residential institution must take place. It is the responsibility of the infection control doctor or hygienist to declare an outbreak and consider the measures outlined in Box 6.3.

Box 6.3 Recommended corrective actions as part of an outbreak investigation

- Shut down any process capable of generating and disseminating airborne water droplets, and keep the system shut down until sampling procedures and any remedial disinfection, cleaning or other intervention have been completed. Final clearance to restart the system may be required.
- Take appropriate samples from the system before any emergency disinfection is undertaken, for use in investigating the source of the outbreak.
- Review and monitor staff health records to find out whether there are any further, undiagnosed cases of illness, and to help prepare case histories for affected people.
- Review water systems records, and investigate any equipment or systems that could have been involved in the outbreak. For example, this may involve tracing all pipework runs, and taking statements from plant operative managers and from water treatment contractors or consultants. Any infringement of relevant legislation may be subject to formal investigation.
- If a cooling water system has been identified as the source of an outbreak of Legionnaires' disease, emergency cleaning of the system should be carried out as soon as possible (see Chapter 5).
- If a water system other than a cooling system is involved in an outbreak of Legionnaires' disease, an emergency treatment of that system should be carried out as soon as possible.

6.5.2 Establish documentation and communication procedures

Table 6.4 gives an example of documentation for a routine monitoring and corrective action loop for a hot and cold-water system (see Chapter 5 for information on corrective action for cooling towers).

Table 6.4 Example of documentation for verification and corrective action for a water system

Process step	Indicator	Monitoring		Operational limit	Corrective action	
Verification	<i>Legionella</i> concentration in water	What	<i>Legionella</i> concentration	In areas for patients with classical individual risk factors, target level of <1000 CFU/l <i>Legionella</i> spp. In some areas for high-risk patients, target level of <50 CFU/l <i>Legionella</i> spp.	What	Raising temperature, disinfection, restriction of water use, use of filtered water
		How	Employ documented, validated and quality-controlled methods		How	Systematic search for failure in the system
		When	2 times/year (4 times/year in high-risk areas)		When	Immediately
		Where	At the entry and at selected point-of-use sites		Who	Plumber (for pump) Building engineer (calorifier)
		Who	Infection control officer or hospital hygienist			

CFU = colony forming unit

6.5.3 Verification

The frequency of verification monitoring of control measures for *Legionella* depends on the status of the system:

- In water systems treated with biocides, where storage and distribution temperatures are lower than the recommended temperatures, samples should be analysed for *Legionella* on a monthly basis. After a year, test results should be reviewed. The frequency of testing may be reduced when confidence in the efficacy of the biocide regime has been established.
- In systems in which control levels are not being achieved consistently through the treatment regime, more frequent samples for analysis of *Legionella* (e.g. weekly) should be taken until the system is brought back under control (see Chapter 3). This action may also form part of a corrective action procedure.
- In hospital wards with high-risk patients, testing for *Legionella* is recommended. The results must be reviewed (HSC, 2000).

Verification requirements for cooling towers are discussed in Chapter 5.

Appropriate diagnostic testing for *Legionella* is necessary and is discussed in Chapter 11.

Chapter 7 Hotels and ships

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This chapter describes how a water safety plan (WSP) can be applied to assessing and managing the risks associated with *Legionella* in hotels and ships.

It should be read in conjunction with Chapter 3, which discusses the different elements that make up a WSP, and shows how a WSP fits within the framework for safe water quality developed by the World Health Organization (WHO).

As explained in Chapter 3, a WSP has 10 steps that fit within the three main areas of system assessment, monitoring and management and communications (see Figure 3.2). A WSP must be comprehensive, and all 10 steps should be implemented in assessing and managing the risks associated with *Legionella*. However, this chapter focuses on parts of the WSP where information specific to hotels and ships is needed.

7.1 Background

The first detected outbreak of legionellosis occurred in a hotel in Philadelphia, United States of America (USA) in 1976. Subsequently, many other cases of legionellosis have been associated with hotels worldwide. Travel and hotel stays are recognized as risk factors for legionellosis (WHO, 1990). In Europe, approximately 20% of detected legionellosis cases are considered to be travel associated (Joseph, 2002b).

7.1.1 European initiatives

Most of the data currently available on the cases of legionellosis associated with travel and hotel stays originate from the European Surveillance Scheme for Travel Associated Legionnaires' Disease, established in 1987 by the European Working Group for *Legionella* Infections (EWGLI). This system — now called EWGLINET — was established principally to enable rapid identification of legionellosis outbreaks among tourists of different nationalities. Its history and current activities are described in detail on the EWGLINET web site.⁵ The need for a specific surveillance system for travel-associated legionellosis in the USA has also been recognized (Benin et al., 2002; Fields, Benson & Besser, 2002).

Cases of legionellosis occurring in hotels have often received extensive publicity in the mass media. Additionally, the growing importance of international tourism, and the significance of morbidity

⁵ <http://www.ewgli.org>

and mortality of hotel-associated legionellosis, justify the attention given to this issue by the tourism and medical community. Since the implementation of the European Community's Directive for Package Travel in 1996, the International Federation of Tour Operators in Europe, together with some tour operators in individual European countries, has been informed of travel-associated cases of Legionnaires' disease in people who purchased holidays through tour operators. The aim of this scheme was to prevent additional cases (Anon, 1996a).

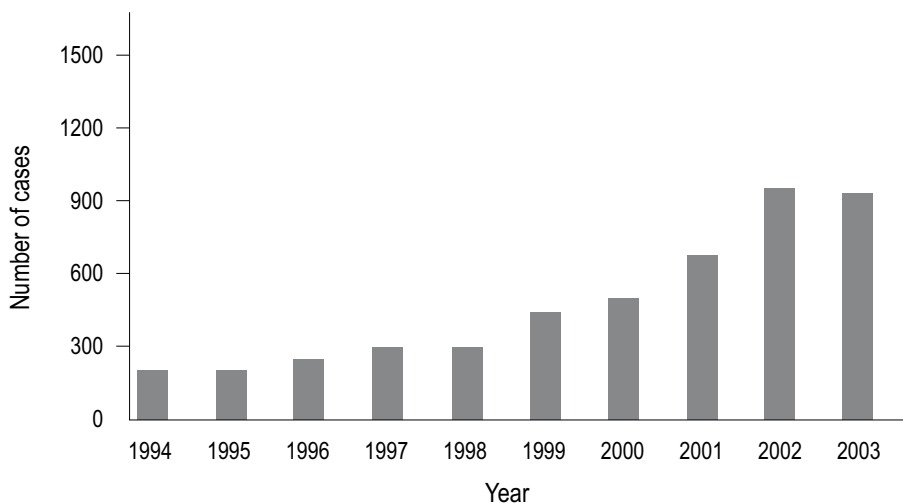
The European tourism industry has developed several initiatives to reduce travel-associated cases (Cartwright, 2000). In some regions and countries with important tourist industries, such as the Balearic Islands (Spain), Portugal and Malta, tourist and health authorities have issued specific recommendations for the prevention of legionellosis in tourist accommodation. In 2002, the *European Guidelines for the Control and Prevention of Travel Associated Legionnaires' Disease* were introduced (EWGLI, 2003).

The number of detected and reported cases of travel-associated legionellosis in Europe rose between 1994 and 2003 (see Figure 7.1). In 1996, travel-associated cases made up 16% of the total number of detected legionellosis cases in Europe; in 1999, they made up 21%. Of these cases, 90% were associated with hotels or apartments; the rest were associated with camp sites, cruise ships, private houses and other sites (EWGLI, 2001).

The sex and age distributions of travel-associated legionellosis cases differ little from those of other cases: they occur mainly in the fifth and sixth decades of life, and with an incidence in men that is approximately three times as high as in women (Ricketts & Joseph, 2004). The mortality rate in travel-associated cases in Europe has dropped over the years, from 10–12% in the early 1990s to 6% in 2003 (Ricketts & Joseph, 2004). This trend probably reflects the improved treatment that follows rapid diagnosis of the illness due to the introduction of the urinary antigen assay (see Chapter 11).

Tourism-associated legionellosis exhibits a clear seasonal distribution that corresponds to the holiday periods usually chosen by older tourists. Most European cases occur between May and November, with the highest peaks in June and September (EWGLI, 1999, 2001, 2004ab). These peaks have been attributed to tourists without school-age children preferring not to holiday during July and August, when the average age of tourists is generally younger, because of school breaks.

Figure 7.1 Detected and reported cases of travel-associated Legionnaires' disease in Europe



Source: Information obtained from the European Working Group for Legionella Infections (EWGLI)⁶

7.1.2 Hotel-associated cases

Although the origin of most sporadic travel-associated cases is unknown, outbreaks have been associated with hotels. Systematic investigation of sporadic travel-associated cases may shed some light on their origin. A major challenge in determining the true source of cases is usually the lack of clinical isolate from the patients. Since the method of diagnosis has moved towards use of urinary antigen detection, it is now rare to have an isolate of *Legionella* from a patient to compare by molecular typing methods with one from the environmental source.

Approximately 60% of hotel-associated cases of legionellosis are sporadic (EWGLI, 2003). The epidemiological relationship between these sporadic cases and the hotel in question is considered to be weak and is often not properly investigated, although it has occasionally been possible to show a causal relationship (Muhlenberg, 1993). When there are several cases, constituting an outbreak, there is an increased probability that the hotel will be the source of infection, and such cases are usually investigated by the health authorities. The epidemiological relationship between clusters and hotels has been corroborated microbially in several outbreaks, including those affecting tourists of different nationalities (Joseph et al., 1996).

⁶ <http://www.ewgli.org/>

7.1.3 Ship-associated cases

Legionnaires' disease was first associated with a ship in 1977 (Rowbotham, 1998). At least 55 incidents (outbreaks or cases) were associated with cruise ships, ferries, cargo ships, fishing vessels or naval ships between 1977 and 2004. Outbreaks of two or more cases are summarized in Table 7.1. Some of these incidents have been linked to ship's water systems, air-conditioning systems and recreational hot tubs. However, in the majority of cases, the source of the infection and/or the mode of transmission were not established.

The risk of exposure to *Legionella* on ships is difficult to assess. Surveys carried out on general cargo ships have shown drinking-water and air-conditioning systems to be contaminated with *L. pneumophila*. Serologic surveys of some seafarers on cargo ships showed that a high proportion had antibodies to *L. pneumophila*, suggesting that those on some ships could be at increased risk of legionellosis compared with communities on shore (Temeshnikova et al., 1996).

The number of outbreaks and cases reported in the literature is probably an underestimate of the true incidence of the disease. As for hotels, outbreaks and cases associated with ships, especially ferries, are difficult to detect because the incubation period of 2–10 days or more (see Chapter 1) means that passengers may have dispersed widely, including to different countries, before developing symptoms. To detect such outbreaks, an international surveillance scheme, such as the European Surveillance Scheme for Travel Associated Legionnaires' Disease, is necessary (see Section 7.1.1).

Even when an outbreak or cluster of cases is detected on a ship or ferry, it is often difficult to implicate that vessel as the source of infection, if passengers disembarked at different locations or stayed in hotels before or after the voyage. Tracking previous incidents associated with ships can also be a problem if the vessel's name has changed, particularly if ownership has also changed. To implicate a particular source during an outbreak investigation, it is necessary to isolate environmental strains of *Legionella* and match them with clinical isolates. However, this is often more difficult to do for ships, because the suspect vessel will often have sailed to another country before a case is recognized. Unless there is good cooperation between international port health authorities and maritime authorities in different countries, ships may escape adequate investigation.

Table 7.1 Review of outbreaks (more than one case) of Legionnaires' disease associated with ships, 1977–2004

Reference	Year of event	Type of ship	Geographical region	Mortality and morbidity	Comments
Christenson et al., 1986	1984	Cruise	Europe and North Africa	• 70 suspected cases • 295/335 passengers had influenza-like illness • 16 hospitalized	• Outbreak occurred after air-conditioning was turned on at Bordeaux • Ship built in 1948
Rowbotham, 1998	1992	Russian training	—	• 4 cases	• Legionellae isolated from water on ship
Rowbotham, 1998	1994	Cruise	Eastern Mediterranean	• 2 cases	• Onsets on the last day of a 15-day cruise and 2 days after a 13-day cruise • <i>L. fallonii</i> and amoebae isolated from the ship
Jernigan et al., 1996	1994	Cruise	USA	• 16 confirmed and 34 probable (1 fatal), over 9 cruises	• Exposure to a spa pool, not adequately disinfected by the brominator, strongly associated with disease
Rowbotham, 1998	1995	River cruise	Rhine River, Germany	• 2 cases, 1 fatal	• Onset 4 days after 7-day cruise
Pastoris et al., 1999	1995	Cruise	Italy	• 3 cases (1 fatal)	• Same serogroup isolated from water supply and from a case
Rowbotham, 1998; Joseph, 1997	1997	River cruise	Germany	• 6 cases (British)	• Spa pool suspected source; <i>L. pneumophila</i> isolated from spa • Chlorine was not applied to the water or monitored

Reference	Year of event	Type of ship	Geographical region	Mortality and morbidity	Comments
Anon, 1998a; Arthur, 1998	1998 May and June	Cruise	Mediterranean and Norwegian fjords	• 3 cases (British)	• <i>L. pneumophila</i> found in hot-water samples from shower heads • Defective temperature control of hot and cold-water systems • Ship had been associated with two other cases since 1995
Cayla et al., 2001	—	Cargo	Spain	• 2 fatal cases	• Two mechanics repairing cargo ship's water system pump • Molecularly indistinguishable strains of <i>L. pneumophila</i> serogroup 1 isolated from patient and pump cooling circuit
Regan et al., 2003	—	Cruise		• 3 cases	• Ship's water supply the source
Lai et al., 2004	2003	Cruise	Iceland	• 8 cases (1 fatal)	• Strains of <i>L. pneumophila</i> serogroup 1 matched patient strain isolated from spa pool and hairdressing station; cases epidemiologically associated with spa pool

7.2 Water safety plan overview

WSPs are increasingly being recommended and implemented in hotels, because testing for *Legionella* in hotel water systems has limited ability to prevent infections. This chapter should be read in conjunction with Chapters 4–6 and Chapter 8, which cover most of the system components found on ships and in hotels.

Developing a WSP for dealing with *Legionella* in hotels and ships involves the following steps:

The remainder of this chapter provides information relevant to a WSP specific for hotels and ships, for each of the three main areas of a WSP:

- system assessment (Section 7.3)
- monitoring (Section 7.4)
- surveillance (Section 7.5).

Sections 7.3–7.5 should be read in conjunction with Section 3.3 from Chapter 3.

7.3 System assessment

This section should be read in conjunction with Section 3.3.1 of Chapter 3. The steps involved in system assessment, some of which are discussed further below, are to:

- assemble a team to prepare the WSP
- document and describe the system (Section 7.3.1)
- assess hazards and prioritize risks (Section 7.3.2)
- assess the system.

7.3.1 Document and describe the system

In addition to piped water distribution systems, *Legionella* has been isolated from many sources in hotels and ships (see Box 7.1). All these sources need to be investigated, documented and described in the system assessment as potential reservoirs of legionellae.

Box 7.1 Potential sources of legionellae to be investigated in a system assessment

Hotels

Potential sources of legionellae in hotels include:

- hot and cold-water storage tanks
- shower heads
- taps
- toilet cisterns
- hot tubs and swimming pools (both cold water and heated pools)
- cooling towers
- air-conditioning humidifiers
- condensation trays in air-conditioners and fan coils
- evaporative coolers
- fire-fighting systems
- irrigation systems
- ornamental fountains
- food humidifiers.

Ships

Potential sources of legionellae in ships include:

- humidifiers (including food display units)
- stagnant areas of pipework
- air-conditioning (suspected) and handling units
- regions on the ship with higher ambient temperatures on board than on shore
- the general complexity of onboard water storage and distribution systems.

Source: Atlas (1999)

7.3.2 Assess hazards and prioritize risks

This step involves collecting and evaluating information on hazards and conditions leading to their presence, to decide which are significant for safety and therefore should be addressed in a safety plan.

A survey conducted in the United Kingdom showed that legionellae were more likely to be found in hotels that had a large number of supply tanks and hot-water outlets, a high-capacity calorifier, and piping made of a metal other than copper (Bartlett et al., 1985). In general, this situation is what would be expected from our knowledge of the ecology of *Legionella* (discussed in Chapter 2).

The risk of legionellosis is increased for those on board ships where legionellae are present in the water systems. Cruise vessels, in particular, have many similarities to hotels in the complexities and operations of their water systems. The risks in ships may also be exacerbated in a number of ways, outlined in Box 7.2.

Box 7.2 Factors exacerbating risks on board ships

- When at sea, a ship is a closed environment, and might provide additional opportunities for the transmission of airborne infection.
- Water storage and distribution systems on ships are complex, and may provide greater opportunities for bacterial contamination as ship movement increases the risk of surge and back-siphonage.
- The risk of sediments in tanks being resuspended and dispersed may be increased by the ship's movement and by adjustments to the water levels in tanks to maintain the trim of the vessel.
- Loaded water may vary in quality and temperature.
- In some tropical regions, the risk of bacterial growth in the cold-water system is increased because of higher ambient temperatures.
- Ships' engine rooms are hot, and may affect water temperatures in pipes passing nearby.
- The movement of the ship could increase the potential for the formation of aerosols (e.g. in air-conditioning ductwork) where there would not be an equivalent risk ashore.
- Proliferation could also result from long-term storage and stagnation of water in tanks or pipes, and this risk could be increased when vessels are laid up for several months.
- *Legionella* can proliferate at temperatures sometimes experienced in stagnant warm water in ships' plumbing systems, especially in tropical regions, and in storage tanks on ships.
- Water can remain in a tank on a ship for a long time in comparison to on land, where storage is usually for less than 24 hours.

Source: Edelstein & Cetron (1999)

Regional aspects — risk factors

Travel-associated infections tend to be diagnosed in the country of residence, because symptoms are often recognized after the patient returns home. The incidence of legionellosis in tourists varies with the country of residence or the outbound country, and the country of infection. The differences may be attributable to differences in diagnostic rates or reporting, rather than to a difference in susceptibility. Historically, the United Kingdom has reported more cases to EWGLINET than other countries, but France, Italy, Germany and the Netherlands have increased their reporting in recent years (EWGLI, 1999, 2001, 2004ab). This increase is probably due to a combination of improved ascertainment (i.e. the determination through diagnostic methodology of whether or not a person is infected with the disease) and improved surveillance. Cases from hotels have also been reported from Japan (Suzuki et al., 2002); Sri Lanka (Wahala & Wickramasinghe, 2000); Beijing, China (Deng, 1993; Peng et al., 2000); Australia (Bell et al., 1996); Serbia and Montenegro (Klismanicacute et al., 1990) and the Caribbean (Schlech et al., 1985).

The rates (per number of tourists) of infection among travellers vary with the country visited. In the period 1997–2002, using the United Kingdom international passenger survey statistics, Turkey was reported as the country with the highest incidence rate, with 10–20 cases per million travellers. Spain, Italy, Greece and France had incidence rates of between one and seven per million travellers (EWGLI, 1999, 2001, 2004ab). Taken together, the countries in southern Europe show higher incidence than those in the north. However, there is a trend towards more declared cases being associated with travel within the home country — in 42% of all the reported travel-associated cases in 1999, infection was related to travel within the country of residence (Joseph, 2002b).

There may be important regional variations within countries (Cano et al., 1999) and also among hotels. Cases of recurrent colonization in hotels have been known for some time (Bartlett et al., 1984). In certain geographical areas (e.g. Benidorm, Spain), a significant percentage of cases have been associated with a small number of hotels (Crespi et al., 1999). Analysis of the data held on the EWGLI database indicated that a hotel previously associated with a case is 15% more likely to have another case than a hotel that has not had a case in the past (Slaymaker, Joseph & Bartlett, 1999). In Spain, of 34 hotels associated with clusters in the period from 1980 to 1999, more than one third (13 hotels) had repeated cases or clusters of cases of *Legionella* on two or more occasions (Martin, Pelaz & Baladrón, 2000).

These data suggest that infections from *Legionella* in hotels are not distributed at random, and that certain hotels tend to transmit *Legionella* persistently. This can sometimes be attributed to a relaxation of controls put into place after an initial outbreak, but in other cases the factors contributing to continuing transmission are unknown.

Hot and cold-water systems — risk factors

Most information about the source of legionellosis in hotels has been obtained from outbreak investigations, which show that the most common source of infection in hotels is the water distribution system, particularly the hot-water system. In Spain, the vast majority of hotel outbreaks in which the source of infection was determined microbially (by showing that clinical and environmental isolates were related) were associated with water distribution systems. In addition, in 12 out of 14 hotels that had subsequent cases after a first outbreak, the origin of the infection was shown to be the hot-water system specifically (Martin, Pelaz & Baladrón, 2000).

The piped water systems of hotels and other tourist accommodation such as apartment hotels are particularly susceptible to colonization by legionellae, because they have large, complex water systems with a high surface-to-volume ratio, and may be subject to seasonal use with long periods of low usage or stagnation. In addition, staff turnover may be high, making it difficult to maintain training and competence.

Legionellae have been isolated from hotel water distribution systems throughout the world. A study of hotels in five European countries (Austria, Spain, Germany, Italy and the United Kingdom) found an average colonization rate of 55%, ranging from 33% in the United

Kingdom to 66% in Spain (Starlinger & Tiefenbrunner, 1996). In Mallorca, Spain, of 114 hotel water systems sampled, 45.6% were positive for *Legionella* (Crespi et al., 1999). A survey in the United Kingdom of 103 hotels between 1982 and 1984 found that *Legionella* was present in 20% of hotels in the north, 43% in the midlands and 52% in the south (Bartlett et al., 1985).

These studies also show that the prevalence of *Legionella* in a water distribution system correlates to a large degree with the water temperature — isolation rates are highest in warm water systems, particularly within a temperature range of 25–50 °C. Starlinger & Tiefenbrunner (1996) also showed a positive correlation between the presence of *Legionella* and amoebae in some installations.

Few published data are available on the concentrations of *Legionella* in the piped water systems of hotels that are colonized but have not been associated with outbreaks. In Germany, Habicht & Muller (1988) detected concentrations of 10^1 – 10^3 CFU/ml in most of the samples analysed, with a maximum of 10^5 CFU/ml.

Hot and cold-water systems on ships have also been implicated in a number of outbreaks. Following an outbreak on a cruise ship in Italy in 1995 and 1996, strains of *L. pneumophila* serogroup 1, identical by monoclonal subtyping and genomic fingerprinting, were isolated from patients and the ship's water supply, although the exact source of the infection was not established (Pastoris et al., 1999). In 1998, an outbreak of Legionnaires' disease occurred on a cruise ship that sailed to the Mediterranean and the Norwegian fjords (Arthur, 1998). Water samples taken from the hot-water system at shower heads were contaminated with legionellae. The ship was unable to maintain safe temperatures in both hot and cold-water systems, and the chlorine dosing system on board the ship was not working effectively (Arthur, 1998). In June 2001, two mechanics working on a cargo ship under repair in Barcelona, Spain were reported to have died after contracting Legionnaires' disease. The mechanics had been working with the pump of the ship's water system. Molecularly indistinguishable isolates of *L. pneumophila* serogroup 1 subgroup Pontiac (Knoxville) were isolated from one of the patients and from the cooling water circuit valve of the ship's pump (Cayla et al., 2001).

Hot tubs and recreational pools — risk factors

Hot tubs are installed on many cruise ships and on some ferries. The risks are similar to those on land (see Chapter 8), and there have been several outbreaks on ships due to hot tubs. In 1994, a cruise ship had 50 cases of Legionnaires' disease, spread over nine cruises. The disease was believed to have been caused by inadequate bromination of the ship's three hot tubs, and the risk of acquiring Legionnaires' disease increased by 64% for every hour spent in the hot tub (Jernigan et al., 1996). Passengers spending time around the hot tub, but not in the water, were also significantly more likely to have acquired infection. *L. pneumophila* serogroup 1 was isolated only from the sand filter of a hot tub (Jernigan et al., 1996).

In 1997, an outbreak occurred on a Rhine cruiser and affected six people. One man had fallen into the cruiser's hot tub and subsequently developed Legionnaires' disease. Large numbers

of *L. pneumophila* were isolated from the pool (Rowbotham, 1998). In 2003, there were eight cases and one death among passengers who had been on a cruise around Iceland. Strains of *L. pneumophila* serogroup 1 that were indistinguishable by multilocus sequence typing were isolated from the hot tub and hairdressing station, but not from anywhere else on the vessel, and infection was epidemiologically linked with the hot tub (Lai et al., 2004). This latter outbreak demonstrates the importance of international collaboration to investigate shipborne outbreaks, since the cases were detected and investigated in Germany after the vessel had docked there to disembark passengers, and it was investigated in its next port of call, in the United Kingdom.

Air-conditioning — risk factors

There are no confirmed reports of outbreaks of Legionnaires' disease associated with air-conditioning systems on ships, but these systems have been suspected in some outbreaks. In 1984, a large outbreak on a cruise ship occurred after the air-conditioning was turned on at Bordeaux, France. No common source was discovered, but the epidemic curve indicated that the air-conditioning system contributed in some way to the outbreak (Rowbotham, 1998). In another outbreak on a cruise ship in 1984, no source was identified, but the outbreak investigation revealed problems with the air handling units (Christenson et al., 1986). Air-conditioning systems on ships are dry and do not have evaporative coolers; however, humidifiers (including food display units) are often installed on ships and could generate aerosols. A study carried out by Temeshnikova et al. (1996) identified *L. pneumophila* serogroup 1 in washings from air-conditioning equipment, and in samples from the mechanical supply and exhaust ventilation equipment on ships.

7.4 Monitoring

This section should be read in conjunction with Section 3.3.2 of Chapter 3. The steps involved in monitoring, some of which are discussed below, are to:

- identify control measures (Section 7.4.1)
- monitor control measures (Section 7.4.2)
- validate effectiveness of the WSP.

7.4.1 Identify control measures

Since the introduction of the *European Guidelines for Control and Prevention of Travel Associated Legionnaires' Disease* in July 2002 (EWGLI, 2002, 2003), the number of hotel cases associated with each identified cluster has reduced, indicating that control measures have been effective in preventing further cases. In 2004, the proportion of clusters involving only two or three cases reached almost 90%, compared with 84% in 2003 and 81% in 2002 (John Lee, Health Protection

Agency, *Legionella* Section, United Kingdom, personal communication, October 2005).

Procedures for control and prevention of hotel-associated legionellosis have been published elsewhere (Crespi, 1993; Anon, 1999; HSE, 2004). The European guidelines give detailed definitions and procedures for responding to travel-associated cases (EWGLI, 2002, 2003).⁷

Ship-associated Legionnaires' disease is preventable. The principles for control of land-based water systems and for WSPs applied to piped water supplies in the engineered building environment (see Chapter 4) are applicable to the control of systems on ships.

Source water quality — control measures

International health regulations require ports to supply potable water to ships; however, there is no requirement for potable water to be *Legionella*-free, and such a requirement would be unrealistic. The water taken on board should be of potable quality and from a reliable source. Since the reliability of the water supply cannot always be guaranteed, precautions should be taken to ensure that the water is adequately disinfected on board.

Hot and cold-water systems — control measures

Primary and secondary methods of prevention and control, as applied to hotels, are based on experience acquired in managing outbreaks and are largely empirical. These measures do not, in general, differ from those that are applied to other types of buildings, in that they aim to eradicate *Legionella* in the installations by means of a risk assessment that focuses on:

- factors leading to *Legionella* proliferation (e.g. the long periods of stagnation that occur in water systems in hotels and hotel rooms)
- implementation of remedial measures (e.g. removal of dead and blind ends, maintenance of elevated temperatures in the hot-water system, periodic disinfection and permanent chlorination of the cold-water system).

The efficacy of these measures in the control and secondary prevention of outbreaks is well established, although they may be insufficient in hotels repeatedly associated with cases. An example of a checklist specifically designed for water systems in hotels is provided in Appendix 1.

In ships, onboard exposure through piped water can be prevented by such water quality management measures as:

- treating source water (where the water is non-potable)
- maintaining water temperatures outside the range in which *Legionella* proliferates (25–50 °C)
- maintaining disinfection residuals greater than 0.2 mg/litre throughout the piped distribution system and storage tanks (WHO, 2004).

⁷ http://www.ewgli.org/public_info/publicinfo_european_guidelines.asp

United Kingdom regulations stipulate that a concentration of at least 0.2 mg/litre free residual chlorine should exist at all outlets, and that water storage tanks should be cleaned at least annually with 50 mg/litre for at least four hours (Department of Transport, 1986), which is often accomplished by supplementary chlorination in the storage tanks and in distribution.

Since it is often difficult to maintain cold water at less than 25 °C on ships, supplementary chlorination is required to maintain disinfectant residual throughout the system; a level of free chlorine will contribute to the control of *Legionella* in such circumstances (WHO, 2004). Water flow in the distribution system should also be maintained during periods of reduced activity.

Disinfection — control measures

A study of 62 hotels in the Balearic Islands, Spain (Crespi et al., 1998) investigated the use of continuous hyperchlorination at 1–2 parts per million (ppm) of free residual chlorine in the cold water, and intermittent thermal treatment in the hot water. Samples positive for *Legionella* dropped from a level of 32.4–31.3%, after the first year of application, to 20% after three years and to 6% after five years. Another study evaluated the systematic purging of the hot and cold-water pipes in two hotels with water chlorinated at 1–1.5 ppm of free residual chlorine (Moreno et al., 1997). Negative cultures were not obtained in the two hotels until five and seven months respectively after the treatment, highlighting the recalcitrant nature of legionellae and the need for repeated and diligent disinfection.

Temperature — control measures

Some buildings may not be able to raise their hot-water temperature sufficiently to control *Legionella* growth; therefore, an on-line treatment such as chlorine or copper/silver ionization should be considered. Chapter 4 has more information on control measures relating to temperature in distribution systems.

Design, operation and maintenance — control measures

The control of *Legionella* in water distribution systems in hotels is difficult, and requires the continuous and effective maintenance of preventive measures.

Hotel personnel responsible for the maintenance of hotel water systems must be educated and qualified to perform these duties. The importance of training and education has been recognized in a large number of published preventive guides. Data from the application of training programmes are very encouraging, and suggest that education may be important in preventing legionellosis in the tourist sector (Crespi & Ferra, 2002).

Given the complexity of distribution systems on ships, the chance of deadends and stagnation is best reduced by proper design of storage tanks and pipes, both at the initial build and when alterations are made, together with a flushing regime if water outlets are not being used regularly. Preventing the risk of colonization during repair of the plumbing systems on ships deserves special attention. Periodic maintenance and cleaning of the water storage tanks (i.e. draining, physical cleaning and biocide treatment) should be carried out at least every six months.

Hot tubs and recreational pools — control measures

The risks from recreational pools and hot tubs on ships can be controlled in the same manner as for pools on land, as described in Chapter 8 and in the World Health Organization *Guidelines for Safe Recreational Water Environments* (WHO, 2006). The Centers for Disease Control in the USA have issued guidelines for prevention of Legionnaires' disease associated with hot tubs on board cruise vessels (CDC, 1997b), and the Health Protection Agency (HPA) and Health and Safety Executive (HSE) in the UK has also produced guidelines for management of hot tubs, which are as applicable at sea as they are on land (HPA, 2006), and update earlier guidelines published by the Public Health Laboratory Service (PHLS, 1994).

Air-conditioning — control measures

Humidifiers or devices likely to amplify or disseminate the bacteria should be periodically cleaned and replaced (Edelstein & Cetron, 1999). Special attention should be paid to the proliferation of *Legionella* in humidifiers. Liquid should not be allowed to accumulate within such units; they must drain freely and be easily accessible for cleaning.

7.4.2 Monitor control measures

Monitoring of systems in hotels and ships, including for facilities such as hot tubs and pools, should largely follow the instructions set out in Chapters 4–6 and in Chapter 8.

Routine monitoring of the water system for *Legionella* has been used extensively in the hotel sector, but its preventive efficacy is debatable; instead, WSPs are increasingly being recommended and used in hotels.

If a source of *Legionella* transmission is identified, especially after an outbreak, a disinfecting procedure (superheating or hyperchlorination) is recommended. Continued maintenance and verification of controls should also be carried out.

7.5 Surveillance

Outbreaks could be detected at a very early stage on ships, if a routine surveillance system for respiratory illness were implemented and if procedures for taking action when the number of cases increases above a certain threshold were followed. As the incubation period of the disease could be longer than the length of a cruise, outbreaks could go undetected, even if the ship has a surveillance system in place. Thus, it is important for community physicians to enquire about recent cruise ship travel if patients present with symptoms of pneumonic illness.

Routine surveillance by external authorities, such as through public health inspections of ships by environmental health officers, should also be conducted, to pre-empt disease outbreaks.

Chapter 8 Natural spas, hot tubs and swimming pools

Susanne Surman-Lee, Vladimir Drasar, John V Lee

This chapter describes how a water safety plan (WSP) can be applied to assessing and managing the risks associated with *Legionella* in natural spas, hot tubs and swimming pools.

It should be read in conjunction with Chapter 3, which discusses the different elements that make up a WSP, and shows how a WSP fits within the framework for safe water quality developed by the World Health Organization (WHO).

As explained in Chapter 3, a WSP has 10 steps that fit within the three main areas of system assessment, monitoring and management and communications (see Figure 3.2). A WSP must be comprehensive, and all 10 steps should be implemented in assessing and managing the risks associated with *Legionella*. However, this chapter focuses on parts of the WSP where information specific to natural spas, hot tubs and swimming pools is needed.

8.1 Background

Bathing has been recognized as a source of infectious disease for centuries; in the 16th century, it was thought that syphilis, plague and leprosy were linked to bathing, and many public pools were closed as a result. Today, there continue to be reports of outbreaks of infectious disease linked to swimming pools, but these can be avoided by:

- good pool management, including adequate filtration and disinfection
- bathers observing advice to shower before entering pools
- bathers refraining from bathing if unwell with diarrhoeal disease.

Immersion in water can be both pleasant and therapeutic, and various techniques have been used over centuries for a diverse range of physiological effects, such as healing injuries, reducing swelling and cooling burns, and for psychological effects, such as calming psychiatric patients (de Jong, 1997). The risk of Legionnaires' disease from swimming pools, spas and hot tubs is low if they are well managed.

This chapter covers swimming pools, spas and hot tubs; Box 8.1 explains what is meant by each of these terms.

Box 8.1 Types of pools

Swimming pools

Swimming pools may be supplied with fresh (surface or ground), marine or thermal water (i.e. from natural hot springs). Pools may be domestic (private), semi-public (e.g. hotel, school, health club, housing complex or cruise ship) or public (e.g. municipal) and they may be supervised or unsupervised. Swimming pools may be located indoors, outdoors (i.e. open air) or both; also, they may be heated or unheated.

In terms of structure, the conventional pool is often referred to as the main, public or municipal pool. It is by tradition rectangular, with no extra water features (other than possible provision for diving), and it is used by people of all ages and abilities. Temporary or portable structures are often used in the domestic setting. In addition, there are many specialist pools for a particular user type — for example, paddling pools, learner or teaching pools, diving pools and pools with special features such as “flumes” or water slides. Although termed “swimming” pools, they are often used for a variety of recreational activities, such as aqua aerobics, scuba diving and so on.

Plunge pools

Plunge pools are generally used in association with saunas, steam rooms or hot tubs, and are designed to cool users by immersion in unheated water. They are usually only large enough for a single person, but can be larger. For the purposes of this document, they are considered to be the same as swimming pools.

Hot tubs

For the purposes of this document, the term “hot tubs” is used to denote various facilities that are designed for sitting in (rather than swimming), contain water usually above 32 °C, are generally aerated, contain treated water, and are not drained, cleaned or refilled for each user. They may be domestic, semi-public or public, and may be located indoors or outdoors. They are known by a wide range of names, including spa pools, whirlpools, whirlpool spas, heated spas, bubble baths or Jacuzzi (a trade name that is also used generically).

Both domestic hot tubs and those in commercial premises have dramatically increased in popularity in recent years; they are now found in sports centres, hotels, leisure and health spa complexes, on cruise ships and, increasingly, in the home environment.

In some countries, especially when in health spa resorts, hot tubs may also be known as hydrotherapy spas or pools, though these terms are more usually applied to pools used within health-care premises (e.g. physiotherapy departments) for treatment that may include swimming (see below).

Whirlpool baths

Whirlpool baths are a type of hot tub often found in bathrooms of hotel suites or private residences. They are fitted with high-velocity water jets and/or air injection but, unlike the hot tubs described above, the water is emptied after each use. They are mainly intended for a single individual, but double versions are available.

Spas

“Natural spa”, denotes facilities containing thermal and/or mineral water, some of which may be perceived to have therapeutic value. Because of their particular water characteristics, natural spas may receive minimal water-quality treatment.

Hydrotherapy pools

In addition, there are physical therapy pools, in which professionals perform treatments for a variety of physical symptoms on people with neurological, orthopaedic, cardiac or other diseases. These are termed “hydrotherapy pools”, and are defined as pools used for special medical or medicinal purposes. Hydrotherapy pools are not specifically covered by this document, although many of the principles that apply to swimming pools and hot tubs will also apply to them. There are also therapy pools containing small fish (*Garra ruffa*) which feed on the scaly skin lesions caused by psoriasis. These types of therapy pools are not covered here.

Source: WHO (2004)

Legionellae have been isolated from swimming pool water and from pool filters (Jeppesen, Bagge & Jeppesen, 2000; Leoni et al., 2001). However, as at 2005, no recorded outbreak of Legionnaires’ disease has been directly associated with bathing in swimming pools. In contrast, hot tubs have been associated with many outbreaks of infectious disease, including Legionnaires’ disease, for which they are the third most common source (Spitalny et al., 1984; McEvoy et al., 2000; Benin et al., 2002; Den Boer et al., 2002; Fields, Benson & Besser, 2002; Nagai et al., 2003). The high incidence of outbreaks associated with hot tubs is due to their increased popularity in recent years. Table 8.1 shows the number of outbreaks related to hot tubs between 2000 and 2003, in selected countries in Europe.

Table 8.1 Reported outbreaks of Legionnaires' disease related to hot tubs between 2002 and 2004

Year	Country/setting	Cases	Source	Notes
2002	Spain	2	H/CWS	Spa centre
2002	Spain	4	H/CWS	Spa centre
2002	Sweden	3	Hot tub	Athletic club
2002	Sweden	23	Hot tub	Hotel
2002	United Kingdom (England and Wales)	3	Hot tub	
2002	Czech Republic	2	Hot tub	Thermal water
2002	Spain	5	Hot tub	Sports centre
2003	Cruise ship	7	Hot tub	
2003	England and Wales	20	Hot tub	Hotel health club
2003	Germany	7	Hot tub	
2003	Sweden	2	Hot tub	Hotel
2003	Spain	4	Not known	An outbreak at a spa
2004	Jersey	2	Hot tub	Holiday apartments
2004	Austria	3	Hot tub	Trade fair

H/CWS = hot/cold-water systems

Source: European Working Group for *Legionella* Infections

It is of some concern that hot tubs, particularly those intended for the domestic market, are commonly found on display at exhibitions and garden centres, where they have not been adequately treated. Just being in the vicinity of a hot tub on display has resulted in cases and deaths due to legionellosis. One of the largest ever outbreaks of Legionnaires' disease, with 21 deaths, was caused by a hot tub on display at a flower show in the Netherlands in 1999 (Den Boer et al., 2002). In the same year, a second outbreak (in Belgium) was linked to a hot tub on display at a fair (De Schrijver et al., 2000). An outbreak of *Pseudomonas* folliculitis, which occurred within two weeks of the installation of a domestic hot tub, was found to be due to the hot tub having been on display before purchase, without appropriate treatment. On investigation, the pool water yielded $8 \times 10^4 - 5.5 \times 10^5$ CFU/100ml *Ps. aeruginosa* and 2.9×10^5 CFU/litre *L. pneumophila* serogroups 2–14. Typing of the *Ps. aeruginosa* isolates from patients and pool showed they were indistinguishable.

Rare cases of Legionnaires' disease have been associated with birthing pool use (Franzin et al., 2001), and one where a spa pool was used as a birthing pool (Nagai et al., 2003) (see Chapter 6).

Various other types of pool are available, such as flotation tanks and small vessels used for therapeutic use. There is no evidence to date of legionellosis associated with these but, as with any water system, the potential for *Legionella* growth within such systems and for aerosol production should be assessed, and an appropriate WSP put in place.

This chapter addresses the risk from infections caused by legionellae in recreational waters. The risks to humans from other infectious diseases and chemicals is dealt with in the WHO *Guidelines for Safe Recreational Water Environments*, Volume 2 (WHO, 2006).

8.2 Water safety plan overview

A WSP needs to be comprehensive; however, an overview of such a plan is shown in Table 8.2, as an example of the type of information a plan might contain. As explained in Chapter 3, a WSP is part of a framework for safe water quality that also includes health-based targets and surveillance.

The remainder of this chapter provides information relevant to a WSP specific for natural spas, hot tubs and swimming pools, for each of the three main areas of a WSP:

- system assessment (Section 8.3)
- monitoring (Section 8.4)
- communication and management (Section 8.5)
- surveillance (Section 8.6).

Sections 8.3–8.6 should be read in conjunction with Section 3.3 from Chapter 3.

Table 8.2 Example of a water safety plan overview for a hot tub (commercial context)

Process step	Pipework	Water in hot tub
Assess hazards and prioritize risks (example) ↓	Stagnant water in deadlegs in the pipework, resulting in proliferation of legionellae	• Sweat, urine, faecal matter and personal care products washed off bathers' bodies in tub, providing a nutrient source for legionellae • Proliferation of legionellae in air-conditioning units in the hot tub room
Identify control measures (example) ↓	Routine cleaning procedures and review of system flow diagram	• Rest period programmed during hot tub operation to discourage excessive use and to allow disinfectant levels to recover • Constant circulation of water in hot tub and replacement of at least half the water in each hot tub at least daily • Signage to encourage bathers to shower before hot tub use and to inform them of proper use of facilities • Filtration monitored by pressure and observation • Cleanliness of hot tub surroundings monitored by observation • Maintaining and physically cleaning heating, ventilation and air-conditioning systems serving the hot tub room (e.g. weekly to monthly)
Monitor control measures (example) ↓	Routine review of process flow diagram to identify areas of concern or stagnation	• Inspection of backwash filters at least daily or on pressure drop • Inspection of cleanliness of hot tub surroundings at least daily (depending on frequency of use) • Testing regime for chemical and microbial parameters (pH and active biocide) at least two-hourly in a heavily used spa in commercial premises
Prepare management procedures (example)	Removal of deadlegs where possible	• Clean or replace backwash filters • Clean pool surroundings • Close facility if necessary
Establish verification and surveillance (example)	• Internal audit and external audit (by the health department) to confirm that operational monitoring and corrective actions are being undertaken as stated in the WSP • Monthly heterotrophic colony counts at the tap and in the source water (to track trends and changes, rather than as an absolute indicator, and to be undertaken by an accredited laboratory) • Three-monthly sampling for legionellae in the pipework	

Process step	Pipework	Water in hot tub
Develop supporting programmes (example)	• Staff training and education; maintenance (including emptying, refilling, backwashing and cleaning instructions) and calibration; response to accidental faeces releases	

Source: Some material taken from HSC (2000)

8.3 System assessment

This section should be read in conjunction with Section 3.3.1 of Chapter 3. The steps involved in system assessment, some of which are discussed further below, are to:

- assemble a team to prepare the WSP (Section 8.3.1)
- document and describe the system (Section 8.3.2)
- assess hazards and prioritize risks (Section 8.3.3)
- assess the system.

8.3.1 Assemble the team

Managing the risk of Legionnaires' disease requires a multidisciplinary approach, including input from:

- designers
- architects
- manufacturers
- installers
- water treatment specialists
- microbiologists
- operatives and users.

It is important that all of these are informed about the potential risks from the systems covered in this chapter.

8.3.2 Document and describe the system

In documenting and describing the system, all relevant information and documentation should be compiled.

Where the design of swimming pools or other recreational water-based facilities includes a water feature created spray (e.g. a fountain), the potential for transmission of legionellae from aerosols should be considered.

System assessments of hot tubs have revealed an array of factors contributing to unhygienic conditions and, potentially, predisposition to legionellae proliferation (see Box 8.2).

Box 8.2 Examples of problems found with balance tanks in hot tubs in commercial settings after a system assessment

Problems identified with balance tanks during investigations of poor microbial quality in hot tubs include:

- tanks found bricked up behind wall
- a tank with a shower built on top of it
- several tanks buried beneath the hot tub, so that access for cleaning is not possible
- tanks constructed of materials that are difficult to clean, such as rough concrete
- some tanks underground, within confined spaces, creating access problems for cleaning
- one tank found to contain large amounts of builders' rubble.

8.3.3 Assess hazards and prioritize risks

This section discusses generic risk factors, in line with the preceding chapters. Where appropriate, and for ease of reference, it also looks specifically at recreational facilities such as hot tubs, although this creates some repetition of information.

Source water quality — risk factors

In pools, the quality of source water is an important factor in preventing microbial growth within the system. Where the source contains high numbers of background heterotrophic microorganisms, or is high in organic content, there is potential for growth of *Legionella* in parts of the water system that may be subject to a rise in temperature (e.g. in storage systems or near underwater lighting or pumps).

Mineral water taken from hot springs is widely used in many spa treatment centres, where it is claimed to be beneficial for relaxation and for its therapeutic effects. The thermal water, which is high in mineral content, is usually drawn from underground boreholes, collected and then distributed. Samples from these boreholes may contain small numbers of legionellae, but high levels have been detected where such water is stored before distribution (Martinelli et al., 2001). As many as seven different *Legionella* species or serogroups have been found in one thermal water distribution system at a spa in the Czech Republic. The high mineral content of these hot spring waters leads to deposition of scale on surfaces in the distribution network, increasing the surface area for bacterial colonization.

Hosepipes may sometimes be used to fill hot tubs and other facilities. If the hose and/or connectors have not been disinfected, the pool may be seeded with nuisance and harmful microorganisms, such as *Legionella* and *Pseudomonas aeruginosa*, which will grow in damp hosepipes left in warm environments.

Nutrients — risk factors

Nutrients for bacterial growth, originating from users of the facilities, are another factor to be taken into account. The turbulence in hot tubs increases the risk of nutrients (e.g. dead skin cells, cosmetics, body lotions and oils) being scoured from bathers. As the water is not drained between users, the nutrients accumulate over the period of use, inactivating biocides and encouraging microbial growth. Many users ignore advice to shower before using pools, increasing the introduction of nutrients, faecal matter and urine.

Hot and cold-water systems — risk factors

A separate and additional risk of legionellosis arises from hot and cold-water systems including showers in the vicinity of a swimming pool (Leoni & Legnani, 2001). Showers should be managed as for hot and cold distribution systems in public buildings, and should be considered in the *Legionella* risk assessment. Chapter 4 discusses risk assessments and control measures for piped hot and cold-water systems.

Disinfection — risk factors

The bathing load, frequency of use and other factors that increase demand on the disinfectant regime must be taken into account at the design stage. For example, hot tubs or natural spas in health facilities that use seaweed therapies or mud treatments may have higher loads of nutrients.

In hot tubs in commercial premises, bathers often override the planned rest intervals (e.g. the water and air jets automatically switching off for 5 minutes after every 15 minutes of use), which would normally allow the hot tub to recover its effective disinfectant potential. The resulting low disinfection residual increases the risk of colonization and growth of bacteria, including legionellae. Bacteria colonizing and growing on surfaces (biofilms) are more resistant to biocides.

Temperature — risk factors

Legionella species can survive, but not grow significantly, in waters at temperatures below 25 °C; however, there is always a risk that legionellae will be present, albeit in small numbers, in the water supplied to pools and their associated water systems (Hsu, Martin & Wentworth, 1984; Ortiz-Roque & Hazen, 1987; Brooks et al., 2004).

Although the risk of *Legionella* growth is reduced in cold-water swimming pools, when water is heated above 25 °C, even in only part of the system, bacterial growth will occur in that region and may then seed the rest of the system.

In hot tubs, the temperature of the water is within the optimal range for the growth of legionellae (30–42 °C).

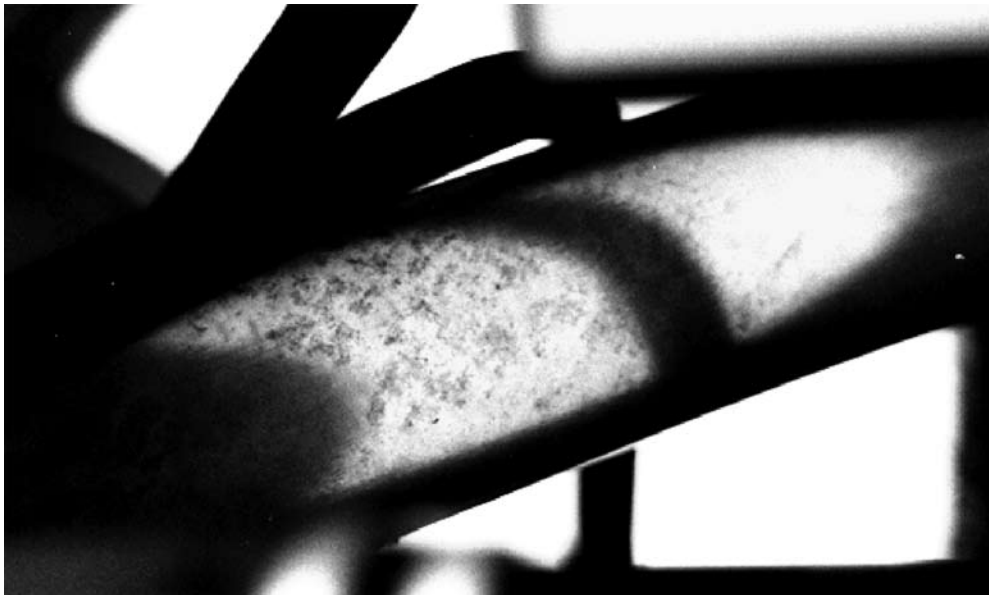
Design, operation and maintenance — risk factors

Thermal water systems, including hot tubs, are at a high risk of being a source of potentially pathogenic microorganisms, including *Legionella*, if they are not designed, installed, managed and maintained with control of microbial growth in mind. This also applies to hot tubs on display, whether or not they are used for bathing.

Hot tubs have a high bather-to-water ratio; they also have an extensive surface area within the pipes used to provide both the air and water-driven turbulence. These pipes are often inaccessible and difficult to clean and drain, and may also have areas of low flow or stagnation allowing biofilms to form as illustrated in Figure 8.1. Pipework above the water line, such as pipework supplying air to the jets, does not usually receive any disinfection from the pool water; its interior is often humid and allows biofilms to form.

Many hot tubs provide no suitable access to all areas of the plumbing system, such as the balance tank, for cleaning, disinfection and maintenance. High levels of legionellae have been found in pools that had areas of pipework with stagnant water (deadlegs) as a result of modifications made to the system.

Figure 8.1 Visible biofilm on internal pipework of a hot tub, two weeks after installation



Photograph courtesy of Susanne Surman-Lee

Hot tubs that are not effectively designed, installed, maintained and managed have a high risk of causing outbreaks of Legionnaires' disease and Pontiac fever, even when not being used for bathing. Further, hot tubs may be sold after having been on display for several months. An important factor for manufacturers to consider is the risk of growth of *Legionella* after construction and leak testing. Residual water left stagnant in the system will grow biofilm microorganisms, which could infect the pool water after the system has been purchased and refilled. Additional risk factors for hot tubs in commercial and domestic settings are detailed in Box 8.3.

Box 8.3 Additional risk factors for hot tubs in commercial and domestic settings

Commercial settings

- Heavy use can result in poor pH control and reduced concentrations of active biocide.
- Staff might not be aware of safety issues because of high staff turnover or short-term employment, or because the hot tub is the only equipment that uses water (especially in small clubs, for example).
- Operators might have insufficient information; for example, they might not know what to do if parameters are tested and found to be outside the acceptable range.

Domestic settings

- Owners often lack information or knowledge about the risks; they often also lack training on treatment and maintenance regimes.
- Domestic spa pools are often located outdoors, where there are no convenient showers. Dust and debris can enter the pool, and windy or breezy conditions can dissipate biocide more rapidly.
- Consistent control can be difficult if the hot tub is used and dosed intermittently.
- A contaminated hosepipe might be used to fill the pool.

Because legionellae have been isolated from whirlpool baths, there is potential for infection (Ishikawa et al., 2004; Susanne Surman-Lee, Health Protection Agency, United Kingdom, personal communication, June 2005). Like hot tubs, whirlpool baths have an extensive array of pipework beneath them, which provides a huge surface area for colonization.

Aerosols — risk factors

Bathers inhale aerosols at a short distance from the water surface, and the high humidity of the environment increases the likelihood of survival of *Legionella* (Berendt, 1980; Hambleton et al., 1983).

Various hydrotherapy treatments occur in some thermal spa resorts, including mouth irrigation, vaginal douches and colonic irrigation. To date, there is no evidence of cases of Legionnaires' disease directly linked to such procedures, but a risk assessment should take into account the susceptibility of the users of such treatments.

Where treatments involve the inhalation of thermal waters, there is an increased risk of exposure to legionellae through inhalation directly into the lungs; nebulizers have been shown to be the source of nosocomial cases (Mastro et al., 1991). Because the use of nebulizers and inhalers involves inhaling fine aerosols, the devices must be filled with water that does not contain potential pathogens such as legionellae, and the suitability of such treatments for high-risk patients must be assessed (see Chapter 6).

8.4 Monitoring

This section should be read in conjunction with Section 3.3.2 of Chapter 3. The steps involved in monitoring, some of which are discussed below, are to:

- identify control measures (Section 8.4.1)
- monitor control measures (Section 8.4.2)
- validate effectiveness of the WSP.

Adequate controls, implemented and maintained in a well-designed and well-constructed system, can ensure the safety of a pool, spa or hot tub. Any control system should be validated and continually monitored to ensure that it works in the pool.

8.4.1 Identify control measures

People who operate hot tubs and pools must fully understand their entire system, and ensure that it is managed and maintained to reduce the risk of exposure to infectious agents. The management structure and staff involved will depend on the nature of the premises. Management systems must be in place to ensure that operators have sufficient knowledge, competence, experience and resources to understand and control the risks of infectious disease, including legionellosis. Inadequate management, poor training and poor communication can all contribute to outbreaks of infectious disease associated with these systems.

Because most hot tubs, spas and swimming pools are operated at temperatures conducive to the growth of legionellae, temperature control cannot be relied on as a control measure in the way it can in distribution and other systems. Therefore, the main control measures are cleaning, operational procedures, disinfection, good source water quality, and maintenance of water quality.

This section should be read in conjunction with Chapter 4, which describes control measures for such factors as source water quality and temperature.

Source water quality — control measures

The starting point for control of legionellae and other microorganisms is to ensure that the water used for filling and topping up the pool is of good microbial quality and free from nutrient sources.

Nutrients — control measures

Bathers have a responsibility to ensure hygienic practices, and should be encouraged to:

- shower before immersion, preferably using soap (adequate signs should be visible, explaining the need)
- adhere to limits set for the number of bathers allowed at any one time
- limit the time spent in the pool.

Ideally, the jet pumps of hot tubs should cut out automatically after 15–20 minutes, so that bathers are encouraged to leave the water and the disinfectant levels allowed to recover (see also *Disinfection* below).

Spa pools should have clearly visible information listing the range of pre-existing medical conditions for which bathing in such pools is not recommended.

Because of the high bather-to-water ratio in hot tubs, it is important to ensure that the water turnover is adequate. Guideline figures vary from six minutes in the United Kingdom (Health Protection Agency, 2006)⁸ to one hour in New South Wales, Australia (New South Wales Health, 1996).⁹

Disinfection — control measures

Choice of disinfectant

The microbial and chemical quality of the water used for filling pools and hot tubs will affect the efficacy of disinfection. Ideally, a detectable residual biocide level should be maintained at all times, to prevent colonization of the system by microorganisms living in biofilms.

Biocides used in hot tubs and pools are commonly oxidizing biocides; for example, chlorine or bromine, sometimes combined with additional treatment regimes such as ultraviolet (UV) light or ozone. Because UV and ozone have no systemic residual effect, they should be used with a residual biocide to improve control and reduce by-products. Alternatively, non-oxidizing biocides, such as polyhexamethylene biguanide and copper/silver ionization (usually with an oxidizing biocide) may be used. Particular features of hot tubs (such as elevated temperatures, high turbulence, high organic load, the amount of sunlight present and natural water chemistry) may affect the choice of disinfectant.

Halogen-based oxidizing disinfectants, such as chlorine, are most commonly used in pools and hot tubs. They have the advantage of being relatively inexpensive, simple to use, easy to measure on site, and active against most infectious organisms. Many commercial and chemical

⁸ www.hpa.org.uk/publications/2006/spa_pools/spa_pools_part1.pdf (accessed 29 March 2006)

⁹ <http://www.health.nsw.gov.au/public-health/ehb/general/pools/publicpools.html> (accessed 12 July 2005)

forms are available (e.g. gaseous, granular, liquid, tablet), with varying amounts of available (free) chlorine, so it is important to follow the manufacturer's instructions carefully. Sufficient disinfectant should be added so that there is still free, active biocide after combination with bacteria, urine and other organic pollutants. The free chlorine residual recommended by the WHO for hot tub water is at least 1 mg/l (WHO, 2006); in the USA, it is 2–5 mg/l (CDC, 2005)¹⁰; in South Australia, it is 2–4 mg/l (Broadbent, 1996)¹¹; in the UK it is 3–5 mg/l (HPA, 2006).

When chlorine is in water it combines with organic materials arising from the bathers, such as urine and perspiration, to form chloramines. These act much more slowly than when chlorine is free or uncombined; they also give rise to odours. Ideally, the level of combined chlorine is nil, but up to a value equivalent of one-third of the total chlorine is acceptable (HPA, 2006).

Bromine-based products such as bromochlorodimethylhydantoin (BCDMH) are often used in hot tubs. When BCDMH dissolves in water, it produces a solution of hypobromous and hypochlorous acid. Bromamines are formed from bromine-based disinfectants in a similar way to chloramines; however, bromamines are still effective as a biocide and are less susceptible to changes in pH. In bromine-treated pools, a residual of 4–6 mg/l of total bromine is recommended.

Ozone is often used in combination with chlorine or bromine; it can be very effective, but it is not suitable for use on its own. Excess ozone is removed by the use of a charcoal filter.

Practical aspects

Features such as water sprays in pool facilities should be periodically cleaned and flushed with a level of disinfectant high enough to eliminate *Legionella* species (e.g. at least 5 mg of free chlorine per litre) (WHO, 2006).

In hot tubs in commercial premises, the introduction of water treatment chemicals should be automatically controlled. Intermittent dosing by hand will not achieve a consistent level of biocide and is not recommended.

The pH value indicates whether the water is acid or alkaline. Maintaining a pH range of 7.2–7.8 for chlorine and 7.2–8.0 for bromine-based and other non-chlorine processes is important for bather comfort, for safety (by controlling disinfectant activity), and for control of corrosion or chemical attack within the pool system. In unusual situations where there is a maintenance fault, the pH could drop to levels at which oxidizing biocides will be disassociated, leading to increased levels of chlorine or bromine, which can cause eye and skin irritation. At high pH levels, the chlorine will remain bound and be less effective.

¹⁰ http://www.cdc.gov/healthyswimming/pdf/spa_operation.pdf (accessed 29 March 2006)

¹¹ <http://www.dh.sa.gov.au/pehs/publications/monograph-heated-spas.pdf> (accessed 29 March 2006)

Various additives may also be used to help maintain the water balance; for example, cyanuric acid helps to stabilize chlorine, particularly in outdoor pools, by preventing its breakdown by UV light and sunlight. Bicarbonates or carbonates may be added to act as a buffer against rapid changes in pH caused by high bather loads, pollutants and chemicals.

In some circumstances, such as in natural spring-fed spas, the addition of chemical disinfectants is considered an adulteration and is not usually allowed, because of the reputed therapeutic effects of the natural water (Martinelli et al., 2001). Since disinfectants are not used, options for control strategies are limited. Pasteurization is the most common means of control, combined with flushing of outlets for 5–10 minutes. The interval between flushes must be based on a risk assessment of the particular system.

Similarly, if mineral water at a hydrotherapy facility is inhaled for its claimed beneficial or therapeutic effects, disinfection might not be considered acceptable, because it would change the chemistry of the water. However, UV treatment may be acceptable, provided the water is not turbid. UV combined with filtration could be used if there is high turbidity.

Design, operation and maintenance — control measures

Systems should be designed, operated and maintained to optimize control strategies. For example, decreasing the available surface area within the system and associated pipework will reduce the potential for bacterial colonization, and avoiding the use of non-metallic materials in construction will help to reduce the risk of *Legionella* growth.

Types and design of materials

Only materials that have been tested and shown to be suitable for use in contact with potable water should be used in the construction and installation of pool, hot tub or spa systems. In choosing materials, their potential to resist microbial growth should be taken into account; this includes not only the surfaces of the pool, but also the materials used for pipes and seals.

Materials used during installation, such as jointing compounds, sealants and washers, should also be considered for their potential to support microbial growth. It is not advisable to use items made from natural materials, such as hemp and natural rubber, because these components promote biofilm formation, as discussed in Section 4.3.2 of Chapter 4 (Niedeveld, Pet & Meenhorst, 1986).

Pipework in hot tubs should have a minimal surface area, which should be smooth so that it does not support colonization by biofilm bacteria. Flexible, corrugated pipework should be avoided, because this increases the surface area and may allow water to be retained in valleys, both of which increase the risk of colonization.

Pipework should also be easily detachable for draining and cleaning — even small volumes of stagnant water will pose a threat from microbial growth in biofilms. There should be adequate access to all parts of the system, including the balance tank and associated pipework. The air and water jets should be removable for physical cleaning and disinfection.

Schedules for cleaning, disinfection and replacement

Treatment regimes should be validated to ensure that they can maintain control of microbial growth under the worst case (highest bather load and throughput), and should be tested when the pool is in use.

Frequent physical cleaning of hot tubs, as well as disinfection, is important because no disinfectant can work efficiently if there is an accumulation of organic matter or biofilms in such areas as the balance tank, strainers, filters and pipework. The whole system, including the balance tank, should be cleaned at least once a week, and sand filters should be backwashed daily. While advice about replacing the water varies, and depends to some extent on the amount of use, a minimum of half the volume of water should be replaced each day (EWGLI, 2003).¹²

Because of problems with rapid build-up of scale in many natural spa facilities, the main distribution pipes are replaced every year. Chemical descaling of pipes is also possible, but is usually considered less cost effective.

For whirlpool baths, it is advisable to disinfect the pipework regularly, while running the pumps intermittently and using a biocide that is approved by the manufacturer as fit for the purpose and that will not damage the surface. Designers should ensure that the system is completely drainable, so that water does not stagnate between uses.

Keeping records

Disinfection, cleaning, operation, maintenance and servicing should be documented in appropriate manuals, which must be integral parts of the risk assessment documents. All pool owners and operators should have available:

- details of the person or people responsible for conducting the risk assessment and managing and implementing the WSP, including their training portfolios
- drawings and updates that accurately describe the system, including any modifications
- operations manuals of manufacturers, suppliers and service providers
- standard operating procedures (e.g. for cleaning and disinfection)
- maintenance and service requirements
- shutdown procedures
- laboratory monitoring reports
- the dates and results of monitoring inspections, tests and checks
- the significant findings of the risk assessment and required remedial measures

¹² http://www.ewgli.org/pdf_files/Guidelines_June_2003.pdf (accessed 12 July 2005)

- descriptions of any significant incidents, their investigation, action plans for necessary work, and changes arising from them.

Records should be retained for several years. These documents should be checked and audited regularly by a competent person, according to the risk assessment.

8.4.2 Monitor control measures

This step involves defining the limits of acceptable performance and how these are monitored.

The pH should be measured continuously in public pools, and adjusted automatically. For hot tubs, monitoring should be conducted several times a day during operating hours (WHO, 2006).

Visual inspection of pool water should accompany other monitoring, such as monitoring of pH and disinfectant residuals. Facilities should be free of visible physical contamination, such as hair, sticking plasters, etc.

Strainers should be inspected and cleared regularly. Where filters are installed, they should be backwashed, either automatically or manually.

8.5 Management communication

8.5.1 Establish documentation and communication procedures

Table 8.3 gives an example of a routine monitoring and corrective action loop.

Table 8.3 Example of documentation for monitoring and corrective action

Process step	Indicator	Monitoring		Operational limit	Corrective action	
Filtration	Particulates	What	Visual contamination (e.g. matted hair)	Absence of gross particulates	What	Physical removal of gross particulates
		How	Visually		How	Clean strainers and flush filters
		When	Daily		When	Immediately
		Where	At the facility		Who	Spa manager
		Who	Spa manager			

8.5.2 Verification

Microbial parameters for hot tubs commonly include the heterotrophic plate count (HPC) at 37 °C, coliforms, *Escherichia coli*, *Pseudomonas aeruginosa*, and sometimes also *Legionella*. For *Legionella*, WHO recommends the following routine sampling frequencies during normal operation (WHO, 2006):

- disinfected pools, public and heavily used — quarterly
- disinfected pools, semi-public — quarterly
- natural spas — monthly
- hot tubs — monthly.

Table 8.4 gives examples of national standards in a selection of countries (Broadbent, 1996).¹³ Well-maintained pools regularly achieve no detectable counts of *pseudomonas*, aerobic colony counts, *Legionella*, coliforms or *E. coli*, and this should be the goal. Failures should be investigated and the effectiveness of any remedial work should be monitored.

Special attention should be paid to microbial sampling for hot tubs linked to cases of legionellosis. In such cases, water samples must be supplemented with swabs from air jets, dismantled shower heads, hoses and taps, including water outlets and inlets. Water samples of 1 litre should be collected from the pool, filter housing and balance tank, where fitted. Balance tank samples are more likely to yield legionellae than hot tub samples; filter material and biofilm from inside the pipes may also contain large numbers of legionellae and should be sampled by swabbing. Often, sections of pipe will have to be cut into to achieve this, but sometimes it is possible to gain access to the insides of pipes by removing the water jets in the base or sides of the spa pool.

8.6 Surveillance

In some jurisdictions, health authorities may periodically inspect facilities (e.g. sports facilities, such as gymnasia); this may include both physical inspections and inspections of records of activities such as cleaning and disinfection. The competency of staff may also be checked; for example, there may be checks to determine whether staff hold appropriate pool maintenance qualifications.

¹³ <http://www.dh.sa.gov.au/pehs/publications/monograph-heated-spas.pdf> (accessed 12 July 2005)

Table 8.4 Examples of microbiological guidelines in legislation and/or guidance for hot tub water quality

Country	Spa whirlpool/ hot tub legislation/ guidance	<i>Legionella</i> limit in hot tubs (CFU)	ACC/ml at 36±2°C	Coliforms/100 ml	<i>E. coli</i> /100 ml	Enterococci /100 ml	<i>P. aeruginosa</i> /100 ml	<i>S. aureus</i> /100 ml	Other
Czech Republic	Decree, Ministry of Health No. 135/2004	<1000/l	100		0		0	0	Nontuberculous <i>Mycobacteria</i> 0/100 ml
Austria	Decree, Ministry of Health BGBl II 1998/420 Baderhygieneverordnung	0/100 ml	100		0		0		
Portugal			<100		0	0	0	0	<i>Staphylococcus</i> spp. <20/100 ml
Spain	Spanish legislation and Basque guidance for <i>Legionella</i> control Basque guidance for spa control	100–1000/l	100	50	0	10	0	0	Other pathogenic microorganisms and parasites absent
Switzerland	SIA Norm 385/1 Edition 2000 (guidance)	0/ml	<1000		0		0		
USA		0							
Germany	DIN 19643	1000/l	<20 (pool) <100 filter effluent		0		0		ACC 20 ± 2°C <20 (pool) <100 filter effluent
Hungary	Statute, Ministry of Health				1		2	2	Total <i>Staphylococcus Micrococcus</i> (7.5% salt)
United Kingdom	HSE / HPA Guidance (HPA 2006)	<100ml	100	0	0		0		

ACC = aerobic colony count; CFU = colony forming units; HPA = Health Protection Agency; HSE = Health and Safety Executive.

Note: Data refer to situations where water temperature is >30 °C and where aerosols could be produced.

Source: Responses to a questionnaire from users of the United Kingdom Health Protection Agency External Quality Assurance Scheme for *Legionella* in Water.

Chapter 9 Disease surveillance and public health management of outbreaks

Carol Joseph, John V Lee

This chapter describes:

- the requirements of a surveillance system for legionellosis (Section 9.1)
- results of an international scheme for surveillance for legionellosis (Section 9.2)
- methods for managing an outbreak of legionellosis (Section 9.3)
- case studies of disease outbreaks (Section 9.4).

9.1 Surveillance systems

Legionellosis is now a statutory notifiable disease in most industrialized countries. Differences in public health surveillance systems mean that provision of *Legionella* data is determined by each country's technical ability to identify cases, produce data and allocate resources to this particular infection. These factors are influenced by the historical, social and cultural value systems that pertain to each country's public health system (Anon, 1998b; WHO, 1999). Thus, a country's national surveillance of Legionnaires' disease will depend on factors such as:

- infrastructure and public health laws
- adopted surveillance principles and standard operating procedures
- notification law
- data protection
- patient confidentiality
- freedom of information legislation.

The priority given to legionellosis surveillance may need to be greater than suggested by local morbidity and mortality, because of its impact on the tourist industry.

Box 9.1 defines disease surveillance.

Box 9.1 Definition of disease surveillance

Surveillance has been defined as:

... the ongoing systematic collection, analysis, and interpretation of health data, essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know. The final link in the surveillance chain is the application of these data to prevention and control. A disease surveillance system includes a functional capacity for data collection, analysis, and dissemination linked to public health programmes.

Source: Adapted from CDC (1996)

9.1.1 Standardized case definitions

Combined microbiological and epidemiological case definitions are used for surveillance of legionellosis. Classifications are shown in Box 9.2.

Box 9.2 Case classifications for legionellosis

Depending on the diagnostic method used and the result, cases are classified microbiologically as either confirmed or presumptive.

Based on the patient's clinical history, cases are classified as one of the following:

- Legionnaires' disease (relevant pneumonic illness and microbiological evidence of infection)
- Pontiac fever or similar illness (relevant non-pneumonic illness and microbiological evidence of infection)
- asymptomatic *Legionella* infection (no illness compatible with microbiological result)
- *Legionella* infection (microbiological evidence of infection but symptoms not known)
- suspected legionellosis (relevant pneumonic or non-pneumonic illness but no supporting microbiological evidence).

9.1.2 Defined datasets

One of the most important pieces of information in the dataset for surveillance of Legionnaires' disease is the history of exposure. The incubation period for legionellosis is normally between two and ten days (see Chapter 1). Thus, whenever possible, an exposure history for two weeks before the onset of illness should be obtained from the patient (or partner, close relative, friend, etc.) to provide a focus for further investigations. A home or work diary and street maps are useful memory aids for this exercise. An example of a two-week exposure history form is given in Appendix 2.

Exposure histories allow cases to be grouped into the following categories:

- community acquired
- domestically acquired
- nosocomial (i.e. health-care acquired)
- travel associated.

For surveillance purposes, cases should be reported to the relevant national centre after the exposure history has been obtained. An example of a national surveillance report form is given in Appendix 3.

Table 1.3 in Chapter 1 provides some useful definitions for epidemiological monitoring at the national level and for comparing exposure risks at the international level; it also defines community clusters and outbreaks.

In the presence of a pneumonic illness, a laboratory diagnosis will support or refute clinical suspicion of *Legionella* infection and will help to classify the case. Table 9.1 summarizes the patient dataset required for surveillance of Legionnaires' disease. The dataset should include as many of the items shown in Table 9.1 as possible.

Table 9.1 Dataset for surveillance of legionellosis

Surveillance dataset	Comment
Demographic history	
Patient age or date of birth	Age is an important moderator for acquiring the disease
Gender	Reported incidence is two to three times higher in men than women
Home address or area of residence	May indicate a local source of exposure
Occupation and occupation address	May indicate an increased risk of exposure
Clinical history	
Date of onset of symptoms for <i>Legionella</i> infection	Relevant to the exposure history and date of specimen for laboratory diagnosis
Other relevant medical history	A recent organ transplant or other causes of immuno-suppression, recent surgery, a history of smoking or high alcohol intake, all increase individual susceptibility
Date and place of hospital admission	Helpful if clinical advice is needed for follow-up of illness outcome
Outcome of illness	Serves as an index of severity and case–fatality ratio

Surveillance dataset	Comment
Exposure history	
Hospital acquired (nosocomial) Date(s) of admission to hospital(s) before onset of symptoms	Necessary to establish nosocomial association
Community acquired Known exposure to cooling towers, whirlpool spas, showers, etc.	Necessary to begin environmental investigations
Travel associated Country visited, dates of stay, name and address of accommodation used, room number, name of tour operator, use of showers, spa pools, etc.	Necessary to begin environmental investigations
Domestically acquired Use of domestic water system during incubation period, in absence of other risk exposures	If no other source of infection identified, this should be considered

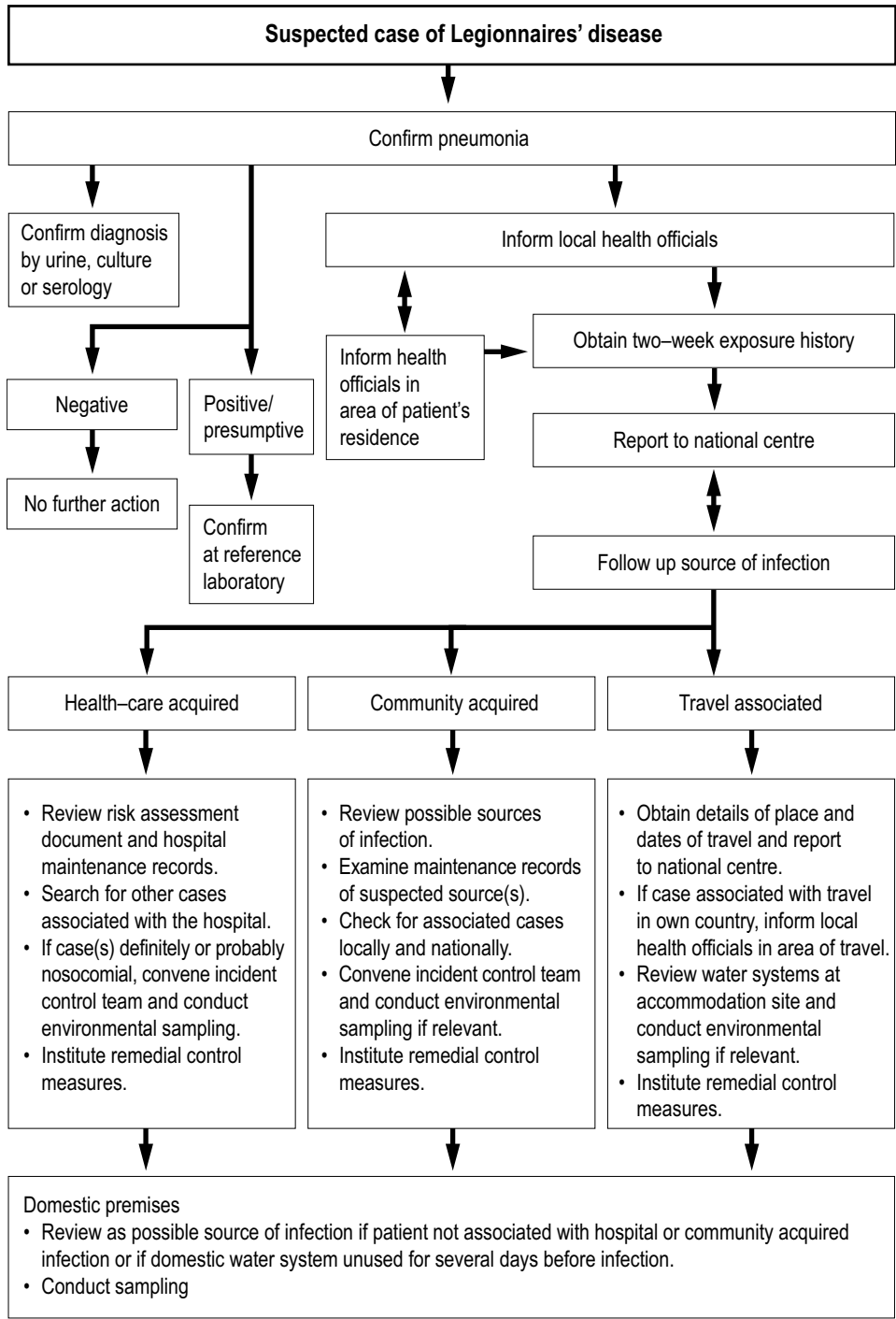
Single cases

Single cases reported to a surveillance scheme are normally entered into a database that is then searched for links in time or place to previously reported cases. If no links are found, the environmental actions in response to a single case will be determined locally or nationally. However, whenever possible, these actions should include:

- a review of the possible sources of infection
- a risk assessment of potential or suspected sources (see Chapter 3).

A memorandum of understanding or a local or national protocol agreed in advance between all the relevant agencies helps to facilitate this process and to ensure that all the appropriate measures have been taken. Local documentation or registration of cooling towers is also extremely helpful when searching for potential community sources of infection. A flowchart for investigating single cases of legionellosis is given in Figure 9.1.

Figure 9.1 Investigating a single case of legionellosis



9.2 International surveillance of legionellosis

National bulletins on public health, published weekly, are important vehicles for disseminating surveillance updates, outbreak reports and notification data from individual countries. Useful publications include:

- *Communicable Disease Report* — published by the Health Protection Agency in England and Wales¹⁴
- *Morbidity and Mortality Weekly Reports* — published by the Centers for Disease Control in the United States of America¹⁵
- *Bulletin of the World Health Organization*.¹⁶

However, interpretation and comparison of surveillance data from different countries can be problematic because of differences in case definitions, types of surveillance system (e.g. national, sentinel, state-funded and private health care) and the types of data collected from cases. The opportunity to carry out international surveillance using consistent definitions and reporting procedures was presented in 1987, when the European Working Group for *Legionella* Infections (EWGLI) established the European Surveillance Scheme for Travel Associated Legionnaires' Disease (EWGLINET¹⁷; see Box 9.3).

Box 9.3 European Working Group for *Legionella* Infections

Since 1993, member countries of EWGLI have submitted annual data to the group by completing a set of standardized reporting forms. These data supplement those provided to EWGLINET, and include the following information on *Legionella* infections:

- annual total cases
- sex group
- numbers of cases, categorized by the exposure group
- main methods used for diagnosis
- species and serogroups of *Legionella* isolates.

¹⁴ <http://www.hpa.org.uk/cdr/>

¹⁵ <http://www.cdc.gov/mmwr/>

¹⁶ <http://www.who.int/bulletin/en/>

¹⁷ <http://www.ewgli.org>

Box 9.3 (continued)

Since the inception of the surveillance scheme, an increasing number of countries have participated. Countries also provide information on the number and type of outbreaks detected each year and the sources of infection (Joseph, 2004b). These surveillance data are extremely useful for:

- comparing incidence rates between countries of similar population size and population density
- comparing the number and size of outbreaks detected
- assessing the effect of national guidance and legislation on the control and prevention of *Legionella* infection in the different countries.

Table 9.2 lists cases of Legionnaires' disease reported to EWGLI in Europe from 1993 to 2004. In 2004, 33 countries provided annual data; their reported cases are summarized in Table 9.3.

Table 9.2 Reported cases of Legionnaires' disease in Europe, 1993–2004

Year	No. of cases	No. of countries contributing data	Population (millions)	Rate per million
1993	1242	19	300	4.14
1994	1161	20	346	3.35
1995	1255	24	339	3.70
1996	1563	24	350	4.46
1997	1360	24	351	3.87
1998	1442	28	333	4.33
1999	2136	28	398	5.38
2000	2156	28	400	5.38
2001	3470	29	455	7.60
2002	4696	32	466	10.1
2003	4578	34	468	9.8
2004	4588	35	557	8.2

Table 9.3 Data on Legionnaires' disease from 33 countries, 2004

Country	All reported cases	Population (millions)	Rate/million
Andorra	8	7.5	1.1
Austria	59	8.1	7.3
Belgium	162	10.4	15.6
Bulgaria	1	8.0	0.1
Croatia (part)	21	1.0	21.0
Czech Republic (part)	15	2.0	7.5
Denmark	103	5.4	19.1
Estonia	5	1.4	3.6
Finland	7	5.2	1.3
France	1201	60.2	19.9
Germany	396	82.5	4.8
Greece	37	11.0	3.4
Hungary	37	10.1	3.7
Iceland	2	0.29	6.9
Ireland	4	3.9	1.0
Italy	561	57.8	9.7
Latvia	0	2.3	0.0
Lithuania	0	3.5	0.0
Luxembourg	8	0.45	17.8
Malta	1	0.4	2.5
Norway	22	4.6	4.8
Poland	13	38.2	0.3
Portugal	60	10	6.0
Romania (part)	2	1.9	1.1
Russian Federation (part)	15	12.0	1.2
Slovakia	1	5.3	0.2
Slovenia	11	2.0	5.5
Spain	984	41.3	23.8
Sweden	109	9.0	12.1
Switzerland	148	7.4	20.8
The Netherlands	242	16.3	14.8
Turkey	9	67.8	0.1

Country	All reported cases	Population (millions)	Rate/million
United Kingdom			
England and Wales	307	52.8	5.8
Northern Ireland	5	1.7	2.9
Scotland	32	5.1	6.3

Part = not reported from entire country

Confirmed cases = 3957 (86.3%); presumptive cases = 575 (12.5%); status unknown = 56 (1.2%)

Source: Information obtained from the European Working Group for Legionella Infections (EWGLI)¹⁸

9.2.1 Effect of improved surveillance

Participation in an international surveillance scheme has led to improved surveillance and higher detection rates in many European countries. For example, Figure 9.2 shows that detection of cases in the Netherlands, France, Italy and Spain has increased with improved surveillance. Figure 9.3 shows reported cases from 1994 to 2004, categorized by type of exposure.

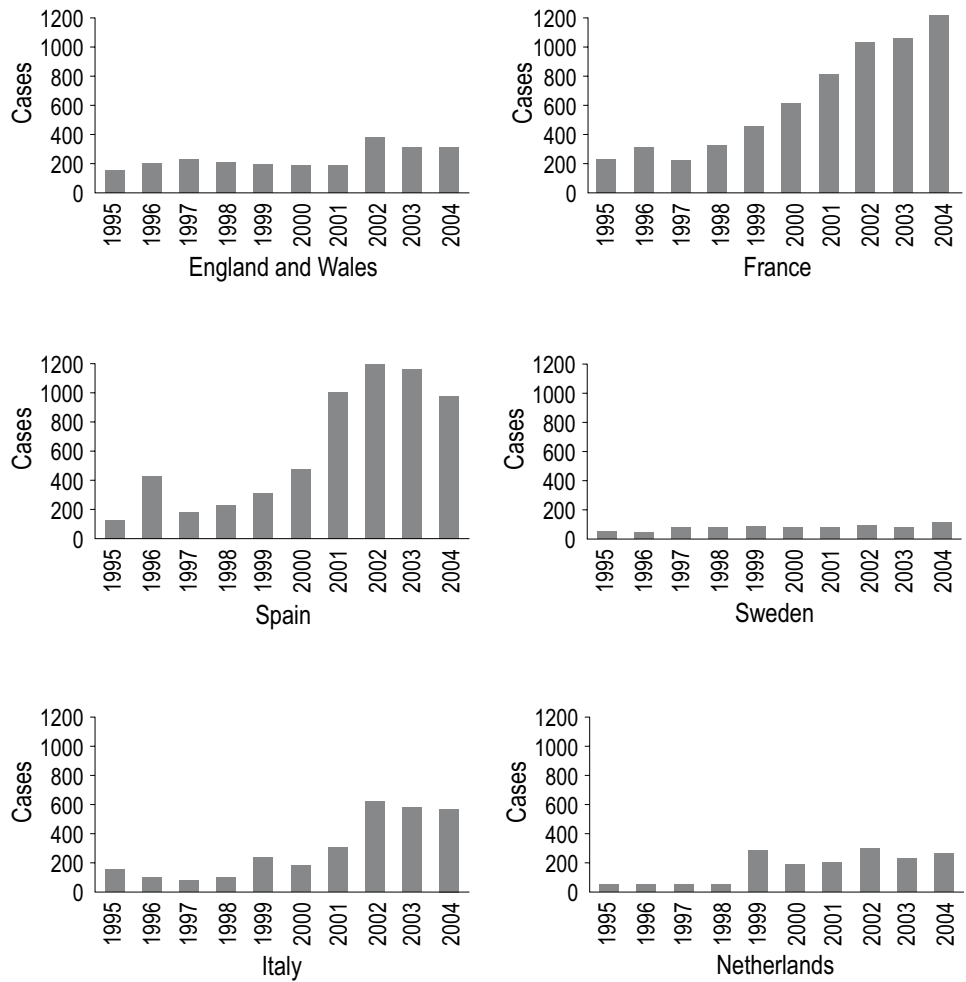
At the national level, underdiagnosis and underreporting are recognized limitations in the surveillance of *Legionella* infections, mainly because:

- many patients with pneumonia are not tested for *Legionella*
- many countries do not have epidemiological follow-up of the laboratory reports through which data are collected and reported.

In Denmark, the annual rate of *Legionella* infections is 17–20 cases per million population, compared with the European average of 4–10 cases per million population. The difference may be due to Denmark's long history of surveillance — the country has a high level of testing for *Legionella* in patients with pneumonia, and a centralized reference laboratory for diagnosing and reporting cases. If all countries had incidence rates similar to those of Denmark, the total number of cases reported by the 33 countries would amount to more than 10 000 per year, rather than the 4500 currently reported. Thus, although the burden of disease associated with Legionnaires' disease is not known, these estimates suggest that it is much higher than is currently recognized.

¹⁸ <http://www.ewgli.org/>

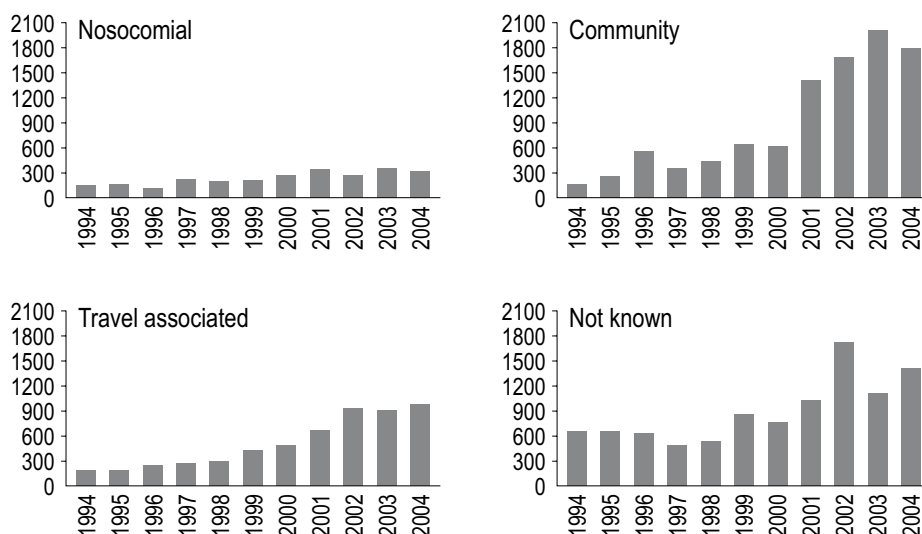
Figure 9.2 Annual reported cases from six European countries, 1995–2004



Source: Information obtained from the European Working Group for Legionella Infections (EWGLI)¹⁹

¹⁹ <http://www.cwgli.org/>

Figure 9.3 Annual reported cases, 1994–2004, by category of exposure



Source: Information obtained from the European Working Group for Legionella Infections (EWGLI)²⁰

9.3 Management of outbreaks

Investigation of an outbreak of Legionnaires' disease is complex, and involves many people from many different agencies. Therefore, clear guidelines and terms of reference must be agreed and practised by all the players involved. By following good public health principles and best practice, the team should operate effectively and be successful in detecting and controlling the outbreak.

This section covers:

- confirmation of an outbreak (Section 9.3.1)
- composition of an outbreak control team (Section 9.3.2)
- policies and practice for outbreak management (Section 9.3.3)
- institutional roles and responsibilities (Section 9.3.4)
- engineering and environmental investigations during an outbreak (Section 9.3.5)
- particular requirements for high-profile disease outbreaks (Section 9.3.6).

²⁰ <http://www.ewgli.org/>

The principles outlined here apply to the investigation of all outbreaks in which microbiological and epidemiological expertise is used, regardless of the disease under investigation.

9.3.1 Confirmation of an outbreak

The first stage in any investigation is to confirm that an outbreak exists. Most outbreaks of Legionnaires' disease will be detected through local or national surveillance schemes.

9.3.2 Outbreak control team

Most outbreaks will be managed by epidemiologists, microbiologists, environmental health specialists and hygienists from the country concerned.

The outbreak control team should be identified and convened before an outbreak occurs. The team must reflect all the relevant organizations responsible for the management of water systems used in industrial, commercial, hospital or leisure facilities, many of whose operations are controlled by legally enforceable codes of practice. A media spokesperson is also essential for preparing and disseminating information to public health officials and the general public, because of the high media interest following detection of an outbreak. Box 9.4 outlines the recommended composition of an outbreak control team.

Box 9.4 Recommended composition of an outbreak control team

An outbreak control team should include at least the following members:

- public health specialists in the area in which the outbreak has occurred
- consultant epidemiologist with expertise in *Legionella*
- consultant microbiologist with expertise in *Legionella*
- environmental microbiologist with expertise in detection and control of *Legionella*
- consultant from the local microbiology laboratory
- environmental health officer or hygienist
- data manager to take responsibility for all aspects of data structure, storage, security and dissemination
- health and safety enforcement officer
- infection control nurse or national equivalent
- representative from the local department of public health medicine
- people responsible for the engineering services at the community, industrial, commercial, hospital or other premises suspected to be associated with the outbreak
- general manager at the community, industrial, commercial, hospital or other premises suspected to be associated with the outbreak
- senior media spokesperson
- other members as decided by the chairperson of the outbreak control team.

Training opportunities

Trainees in public health medicine, epidemiology, microbiology or environmental health welcome the opportunity to become involved in outbreak investigations. However, their training activities role should not hinder the work of the outbreak control team, and trainees should not be put into roles with responsibilities beyond their level of expertise or competence. The trainees should be a resource for the outbreak control team at a level appropriate to their training needs.

9.3.3 Policies and practices

Good public health practice must be paramount when planning the management of an outbreak of Legionnaires' disease. International travel-associated outbreaks will be managed differently (see below).

The outbreak control plan should outline the local or national lines of communication in response to the diagnosis of one or more linked cases of legionellosis. The plan should specify who is responsible for convening the outbreak control team, and the key groups of staff to be included in the team.

Once an outbreak is suspected or confirmed, the control team should be convened immediately. At its first meeting, the team should:

- elect a chairperson, who will be responsible for convening all future meetings and organizing secretarial support for taking and promptly distributing minutes and any other information associated with the outbreak
- establish terms of reference for the outbreak investigation (see Section 9.3.4)
- determine which groups of public health professionals will be enforcement or legislative authorities for prevention and control of *Legionella* infection
- agree on a plan for ensuring that immediate action is taken to eliminate the source of infection, once it has been identified
- review epidemiological information to decide where to focus the initial environmental investigations and control measures (see Section 9.3.5).

Resources

Regardless of the size, magnitude and duration of an outbreak, it is vital that sufficient resources are mobilized and maintained until the investigation is complete. This includes resources for managing all aspects of the outbreak investigation, analysing data and producing an outbreak report.

European guidelines

European guidelines for the control and prevention of travel-associated Legionnaires' disease came into use in July 2002. These guidelines, produced by EWGLI, were endorsed by the European Commission in June 2003. The guidelines formalize the procedures for responding to clusters of Legionnaires' disease in the country of infection.

Within two weeks of the cluster alert, the collaborator in the country of infection is required to inform the hotel, arrange for an immediate risk assessment, and arrange for control measures to be implemented. Within six weeks, the results of a full environmental investigation must be reported, including the results of any sampling that has taken place and information on whether the hotel remains open or closed. If this information is not received within the specified time, or control measures are found to be unsatisfactory, the name of the hotel associated with the cluster is posted on the EWGLI web site, where it remains until the relevant information is received at the coordinating centre.

The average number of cases in a travel-associated outbreak has declined in the past two years, because of rapid and effective interventions by the participating countries.

International travel-associated outbreaks

Occasionally, countries will request international collaboration, as happened after travel-associated outbreaks in Turkey (Joseph & Lee, 1996; Brand et al., 2000), Antigua (Hospedales et al., 1996), Spain (Garcia-Fulgueiras et al., 2003) and elsewhere. Increasingly, more than one country may participate in an investigation through exchange of clinical and environmental specimens or sequence typing data from an outbreak (Joseph et al., 1996; Gaia et al., 2003). International collaborations help to validate diagnostic tests and the microbiological association between cases and sources of infection.

A major outbreak of legionellosis, particularly an outbreak considered to have international public health importance, would warrant notification under the International Health Regulations (2005) and, when requested, a WHO coordinated response, including support to the affected country and information to alert other countries of a potential health threat.

9.3.4 Roles and responsibilities

The control team should have terms of reference that clarify the roles and responsibilities of the relevant partner agencies and disciplines, and that cover all identified tasks. This is critical to the smooth management of an outbreak. A sample checklist is given in Box 9.5, and further information on some of these issues is given below.

Box 9.5 Example of terms of reference for an outbreak control team

The terms of reference for an outbreak control team should include at least the following areas:

- membership and composition of the team
- allocation of tasks
- confidentiality and ownership of data
- disclosure and dissemination of information
- preparation of reports — immediate, interim and final
- authorship of publications
- review of outbreak procedures, management and outcomes
- documentation of lessons learnt.

Confidentiality and information disclosure and dissemination

Confidentiality of data should be respected at all times; therefore, information on cases received in medical confidence should be confined to members of the outbreak control team and should be referred to without patient identifiers when reports are produced for wider dissemination. Media reports should also respect the confidentiality of the data on which they are based.

Procedures for disseminating information from the outbreak investigation should be agreed in advance, so that all relevant people are aware of the latest findings and developments in the investigation. All members of the outbreak control team should be prepared and informed through regular telephone conferences. When results of laboratory findings are being released, or testing of specimens is being requested, the channels of communication should be made clear, so that the appropriate people are informed in the correct order. Normally, the chairperson will receive the results of all diagnostic tests and forward them to the relevant members of the outbreak control team.

Outbreaks generate a great deal of anxiety among the population involved; there is often extensive media coverage, and the outbreak control team may be subject to excessive public scrutiny during the course of the investigation. A media spokesperson or a single member of the team should therefore be designated to speak to the media to ensure consistency. It is a good idea to have a pre-prepared press statement.

The media can sometimes be used to help find cases and protect public health by providing advice. Questions and answers can be pre-prepared and posted on the internet. For example, the web site of the United Kingdom's Health Protection Agency provides general information on Legionnaires' disease in the form of questions and answers.²¹ The site poses and answers questions such as "What is Legionnaires' disease?", "Why is it called Legionnaires' disease?", "How is Legionnaires' disease spread?" and "What are the symptoms?".

²¹ http://www.hpa.org.uk/infections/topics_az/legionella/gen_info.htm

Preparation of reports

The outbreak control team should produce regular reports on the outbreak investigation. They should also produce a final report for dissemination to members of the team, the ministry of health or equivalent government agency, the chief executive of the health authority or the region where the outbreak took place, and any other relevant institutions.

Investigation records or documents held by the outbreak control team may be required if litigation arises out of a demonstrable breach of practice in the operation or maintenance of aerosol-generating water systems.

Review of outbreak procedures, management and outcomes

When the outbreak is over, the final meeting of the outbreak control team should include a review of the way the outbreak was managed and any lessons learnt from the investigation process. This information should be included in the final report produced by the team. If a report for publication in a peer-reviewed journal has been discussed, the chairperson should review authorship with the team and agree on the principal authors before producing the publication. Any outstanding litigation or criminal proceedings that might prevent publication of certain findings from the investigation must be considered before going ahead with a report for publication.

9.3.5 Engineering and environmental investigations

Obtaining environmental isolates

In all outbreak investigations, it is important to prevent further cases and ensure that the source has been located. This can be achieved by obtaining environmental isolates, which can then be matched with those of the patients (if available). Hence, wherever possible, potential sources should be sampled before any precautionary disinfection. In many cases, equipment can be made safe simply by switching it off or not using it; for example, fountains can be switched off and showers temporarily closed until after sampling and disinfection. With non-essential pieces of equipment, it may be possible to leave the equipment out of action until microbial analyses are complete and there is confirmation either that the equipment is not contaminated or that it has been successfully decontaminated.

Target of investigations

As explained above, the outbreak control team first reviews the epidemiological information to decide where to focus initial environmental investigations and control measures. If the patients are all associated with a particular building, the initial investigations should be targeted at all the water uses (as described in Chapters 4–8) in that building. Investigations of the piped water system should include the rooms used by the patients, as well as the systems as a whole. Ideally, the water systems should be subjected to a risk assessment; however, in the initial intensive phases of an outbreak investigation, a brief, rapid assessment is often all that is possible,

because doing anything more could unduly delay the collection of samples and the initiation of control measures. Thus, the initial risk assessment is often necessarily superficial, but is often followed by a more complete assessment once the initial intensive sampling phase is over.

Potential sources outside the building

Even when the initial epidemiological evidence indicates a particular building as the source, the possibility of a source outside, but close to, the building should also be considered. In the United Kingdom, investigations have usually concentrated on all potential sources within a 500-m radius of the epicentre of an outbreak, although cooling towers and evaporative condensers are inevitably the most likely targets. Such investigations are aided if the local authorities have a register of cooling towers in their area (a requirement in the United Kingdom; Anon, 1992).

All cooling towers should be visited as soon as possible and sampled before being given a precautionary disinfection with a high dose of chlorine (50 mg/litre for at least 1 hour) or another suitable oxidizing biocide. As further epidemiological evidence becomes available, the epicentre of the investigations may shift and other water systems may need to be targeted. Once all potential sources within the 500-m radius have been identified and visited, the radius may be increased to 1000 m or more. Transmission is usually only considered likely up to about 2000 m, although in an outbreak in Lens in the north of France in 2003–2004, transmission up to 8 km has been suggested (Nguyen et al., 2006).

It is usually easiest to investigate each water system systematically by starting at the water supply into the property and working forwards through storage tanks and any intermediate equipment, such as water heaters and softeners, to the outlets.

Changing epicentre

The epicentre of an investigation may change rapidly during an outbreak. For example, in 1999, the initial investigation of an outbreak in Piccadilly Circus in London centred on a hotel several hundreds of metres to the south, because the first two cases recognized stayed there. The hotel was investigated and sampled during the night following the day on which the first cases were reported. On the following day, as more cases were discovered, the focus shifted to Piccadilly Circus (Watson et al., 1994; JV Lee, Health Protection Agency, United Kingdom, personal communication).

Large numbers of sources and samples

The investigation of an outbreak can be extremely labour intensive, particularly:

- in city centres, where there may be many tens or even hundreds of cooling towers within a few square kilometres
- on industrial estates, where there may be many and varied potential sources.

The number of samples collected can be considerable, and it will often be necessary to use more than one laboratory to ensure that all the samples are processed in good time. If this is the case, the outbreak control team should ensure that the laboratories used are competent and experienced, and use the same method of detection, with the required sensitivity. The outbreak plan, which should be prepared in advance, should include information on:

- roles that individuals may play
- laboratories that are to be used
- contingency plans for situations in which the local laboratory cannot cope
- means to rapidly obtain sufficient laboratory media
- transport arrangements for specimens, to ensure arrival within the recommended time.

Chapter 11 has detailed information on sampling for legionellae. Advice on sampling that complies with the European and United Kingdom guidelines has recently been published and is freely available from the Internet (Standing Committee of Analysts, 2005).

9.3.6 High-profile outbreaks

Occasionally, outbreaks may be of such magnitude or importance that all investigations should be managed from an incident room established within a national public health institution. In such cases, resources must be identified, because many staff may be required for:

- interviewing patients
- carrying out a case-control study to determine the epidemiological importance of certain risk factors
- collecting and processing clinical and environmental samples.

These staff will not be part of the outbreak control team but must be briefed regularly to ensure that all resources required for the outbreak investigation are used efficiently and effectively. Regular updates of factors such as case ascertainment, patient outcomes and environmental results must be conveyed to all core members of the outbreak control team. This does not necessarily mean convening meetings; it can be achieved using e-mail groups, video conferencing or telephone and fax communications by the chairperson of the outbreak control team.

Where there is an international dimension, the relevant health departments in overseas governments, the relevant department in WHO,²² and other stakeholders and institutions must be informed. Case searching and follow-up must be organized through national public health institutions.

²² outbreak@who.int

9.4 Case studies

This section describes:

- a community outbreak in England (Section 9.4.1)
- a health-care facility outbreak in Israel (Section 9.4.2)
- an outbreak associated with hot tubs in Austria (Section 9.4.3)
- a case of Legionnaires' disease associated with a concrete batcher process on a construction site in the UK (Section 9.4.4).

9.4.1 Community outbreak — England

In late 2003, 27 cases and two deaths were associated with an outbreak in a small city in England. As soon as the outbreak was recognized, the outbreak control team was convened. The team constructed a case definition, and carried out detailed epidemiological and environmental investigations.

The source of the outbreak was shown to be the cooling towers at an industrial plant used to make cider. The industrial process involved switching on the cooling towers once a year, when the apples used to make the cider were delivered to the plant for processing.

None of the workforce became ill, but clinical isolates obtained from two of the cases were indistinguishable by sequence-based typing methods from the environmental isolates obtained from the cooling tower water samples (Gaia et al., 2003).

The investigation included use of meteorological data, plume modelling, helicopter infrared surveillance of potential sources of infection, and geographical information systems for analysis of patient travel in the local vicinity over the outbreak period.

The outbreak was stopped when the cooling towers were shut down (Anon, 2003).

9.4.2 Nosocomial outbreak — Israel

During a two-week period in June–July 2000, a nosocomial outbreak of *Legionella* pneumonia caused by *L. pneumophila* serogroup 3 occurred in four patients, following haematopoietic stem cell transplantation, in a new bone marrow transplant unit. The causative organism was recovered from the water supply system to the same unit, just before the outbreak occurred. Serologic screening revealed no other cases of *Legionella* pneumonia in 19 consecutive bone marrow transplant patients hospitalized in the same unit at the same time.

The outbreak was contained by early recognition, immediate restrictions of the use of tap water, antibiotic prophylaxis for all non-infected patients, and water decontamination by hyperchlorination and superheating. In November 2000 and February 2001, two more nosocomially acquired cases occurred, along with the re-emergence of *Legionella* in the water.

This case highlights the high risk for *Legionella* pneumonia among bone marrow transplant patients, and the need to take permanent (rather than intermittent) decontamination measures to prevent nosocomial *L. pneumophila* in high-risk patients (Oren et al., 2002).

9.4.3 Hot tub outbreak — Austria

In March 2004, a spatial and temporal cluster of cases of legionellosis occurred in a small area of northern Austria. The cluster prompted immediate epidemiological and environmental investigations by the Austrian Agency for Health and Food Safety. Four cases of *L. pneumophila*, with onset of illness between 10 and 13 March 2004, were reported to the Austrian Legionella Reference Centre by hospital laboratories or local health authorities. The cases were all male and were aged between 28 and 65 years.

In all four cases, pneumonia was diagnosed clinically and by X-ray, and all cases had a confirmed laboratory diagnosis by detection of *L. pneumophila* antigen in urine. A significant seroconversion (more than fourfold) to *L. pneumophila* serogroup 1 was observed in the first and third cases. The reference laboratory detected *L. pneumophila* serogroup 1 by direct fluorescent antibody staining, and *L. pneumophila* DNA (deoxyribonucleic acid) by polymerase chain reaction in the respiratory secretion of the third case. A single, high-specificity serum antibody titre to *L. pneumophila* serogroup 1 was found in the fourth case. No isolates were obtained from any of the cases.

All four patients were hospitalized. The third case, a 65-year-old patient, developed multi-organ failure and required mechanical ventilation and haemodialysis for 11 days. All cases recovered.

The Federal Ministry for Women and Health announced this cluster of cases of *Legionella* infection in a press statement on 31 March 2004. The ministry initiated active case finding by alerting practitioners and clinicians working in the areas where the cases occurred.

The four cases were linked by area of residence. The timing of clinical onset indicated that all were exposed to a common source of infection during a restricted period. Interviews with the patients about their activities during the 10 days before clinical onset revealed that all had attended a trade fair for energy-saving products, held on 5–7 March 2004 in a city near their residences. The trade fair included hot tub display stands. All patients reported that they had visited the hot tub stands at the exhibition.

This information prompted a series of environmental investigations. Water samples were obtained from the cold and warm water system of the exhibition centre at which the trade fair was held. Only 5 out of 20 demonstration hot tubs that had been exhibited at the trade fair were identified and sampled. No legionellae were detected in any water sample.

The epidemiological evidence indicates that the most likely source was one or more hot tubs at the display. The fact that the microbiological environmental investigations did not confirm this was probably due to the inevitable delay between exposure and the investigation.

9.4.4 Concrete batcher process on a construction site — UK

This section is based on a case study submitted by SB Surman-Lee, C Seng and T Harrison, of the Health Protection Agency, London, UK.

Untreated warm water and high pressure aerosols are high-risk factors for causing Legionnaires' disease. Aggregate (used in making concrete) stored outside in winter in the UK is too cold for production of some concrete mixes. A case of Legionnaires' disease was found in a construction site worker. The person was working near a concrete batching plant where warm water (about 30 °C) was added to a concrete batcher to facilitate the chemical process during cold weather. The untreated warm water source was a storage tank containing borehole water, heated by an adjacent boiler.

A powered jet washer connected into the warm water supply was used to hose down and remove concrete from the batcher plant, surrounding areas and lorries. Water from the storage tank, associated pipework and jet washer had high levels of *Legionella pneumophila* serogroup 1 (>105 CFU/litre). Isolates obtained from the patient and the environmental sources were found to be indistinguishable by further typing from *L. pneumophila* serogroup 1, MAb subgroup "Knoxville", SBT 3,10,1,10,14,9.

This is the first time that a case of Legionnaires' disease has been associated with concrete production on a construction site. The site workers believe that similar systems operate elsewhere. This case therefore highlights the need for a thorough risk assessment of all systems using water on construction sites worldwide, and the need for systems to manage and control warm water used in similar processes.

Chapter 10 Regulatory aspects

David Cunliffe

Two complementary types of regulatory approach can be applied to legionellosis:

- preventing risk from systems that can support the growth and dissemination of *Legionella*
- notification of illness.

The water safety plan (WSP) approach (WHO, 2004) provides an appropriate mechanism for implementing preventive risk management systems, and should form the basis of guidelines or regulations developed for controlling *Legionella*.

WSPs incorporate multiple barriers; in the case of *Legionella*, this approach should focus on the events that, combined, are prerequisites for most waterborne *Legionella* infections. These events include:

- survival and growth of virulent organisms
- inhalation of aerosols
- aspiration
- exposure of susceptible hosts.

Each of these factors can be influenced by management practices, and hence can be subject to regulation.

Disease notification systems provide a basis for initiating investigations, identifying sources of infection, issuing public advice and limiting the scale and recurrence of outbreaks. Notification and investigation systems can be incorporated within regulations.

10.1 Existing guidelines and regulations for risk prevention

Many countries have developed guidelines or regulations for the control of *Legionella* in water systems and for the prevention of legionellosis. Guidelines are advisory, whereas regulations and codes of practice have a more formal standing and are supported by legislative enforcement (including, in the case of regulations, specific information on managerial responsibility and operator competency).

- Guidelines include NHMRC (1988); CDC (2003); Allegheny County Health Department (1997); ASHRAE (2000); Ehrlich, Steele & Sabatini (2000); Standards Association of Australia/Standards Association of New Zealand (2002); EWGLINET (2003); and WHO (2004, 2006).

- Regulations include HSC (2000), IEE (2001) and Victorian Department of Human Services (2001). Table 10.1 summarizes European regulations.

WHO publications relevant to the control of *Legionella* are listed in Box 10.1.

Current guidelines and regulations vary in scope and design, but usually include certain common features, such as general support of a risk management approach. Some guidelines are very broad (e.g. ASHRAE, 2000; HSC, 2000), whereas others deal with specific circumstances, such as control of infection within health-care facilities (Allegheny County Health Department, 1997; Ehrlich, Steele & Sabatini, 2000; CDC, 2003) or travel-related disease (CDC, 1996, 1997a; CDC, 2003; EWGLINET, 2003).

Box 10.1 WHO publications relevant to the control of *Legionella*

- *Guidelines for Drinking-water Quality*, third edition (WHO, 2004)
- *Guidelines for Safe Recreational Water Environments*, Volume 1 — *Coastal and Fresh Waters* (WHO, 2003)
- *Guidelines for Safe Recreational Water Environments*, Volume 2 — *Swimming Pools, Spas and Similar Recreational Water Environments* (WHO, 2006)
- *Health Aspects of Plumbing* (WHO, 2006)
- *Legionella and the Prevention of Legionellosis* (WHO, 2007)
- *Guide to Ship Sanitation* (WHO, 2007)

10.2 *Legionella* testing

An issue that has been the subject of some debate is the role of testing programmes for the presence or absence of *Legionella*, and whether such testing should be included in regulations. Chapter 11 discusses some of the issues involved in such testing programmes, which include lack of correlation between test results and human health risk (Kool et al., 1999; Bentham, 2002), and uncertainties about whether or not detected legionellae are infectious (Bentham, 2000).

Much of the debate about routine testing for *Legionella* has focused on the potential for over-reliance on results at the expense of risk management strategies. *Legionella* testing is not suitable for operational monitoring, in the same way that enteric pathogens and indicator bacteria are not suitable for operational monitoring of drinking-water supplies (WHO, 2004). However, *Legionella* testing can be a useful component of monitoring to verify the performance of water safety plans (WSPs), and it is recommended for cooling towers, hot tubs and water distribution systems where people at high risk might be exposed (e.g. in health-care facilities).

Legionella testing can also be undertaken as part of:

- investigation of an outbreak

- validation of the effectiveness of control measures
- verification of the effectiveness of decontamination.

If requirements for testing are included in regulations, it is important to define the purpose of testing and how results will be treated.

10.3 Scope of regulations

Regulations for control of *Legionella* should be framed within a preventive risk management approach that is consistent with the approach outlined in WSPs and the *Framework for Safe Drinking-water* (WHO, 2004). Preventive risk management is based on the premise that it is far better to prevent hazardous situations occurring than to wait until they occur and then take remedial action.

For operational monitoring, risk management relies on measuring parameters that show whether systems are working properly, rather than relying on end-point testing, which often only shows whether a system worked at some earlier time.

Risk management strategies for *Legionella* should incorporate a multiple barrier approach aimed at controlling the growth, survival and dissemination of the bacteria. Multiple barriers have long been used to deal with waterborne organisms. Although all barriers should preferably remain functional at all times, one advantage of the approach is that if one preventive measure fails, others may maintain adequate protection.

Specific matters that should be covered by regulations include:

- managerial responsibilities and reporting requirements
- system assessment of buildings and devices that are potential sources of *Legionella* (e.g. cooling towers, water distribution systems, spa pools, humidifiers, ice machines); this assessment should consider the susceptibility of those who may be exposed (e.g. transplant and cancer patients) and those who are immunocompromised or receiving immunosuppressive treatment
- control measures to prevent the growth, survival and dissemination of *Legionella*
- operational monitoring procedures to ensure that control measures remain functional whenever devices are in use
- verification procedures to ensure that WSPs are operating effectively
- mechanisms for surveillance and audit of risk management plans.

Regulations could also include requirements relating to notification of disease and responses to outbreaks of disease.

10.4 Designing regulations

Basic principles need to be observed when developing new regulations. There needs to be an overall aim, which in this case is to reduce the risk and incidence of legionellosis. The aim is achieved by applying a set of specific requirements, each of which should have a specific and stated purpose. Compliance with requirements should be measurable and (where appropriate) enforceable. Monitoring regimes should be defined, and corrective action to remediate non-compliance should be described.

Specific health-based targets might be established for the incidence of illness or outbreaks. Such targets can be useful in undertaking cost–benefit analyses as part of a regulatory impact assessment. If *Legionella* testing is included in requirements, target concentrations and responses to detections should be specified.

In some cases, there may need to be a balance between different regulatory requirements. For example, in the case of water distribution systems, avoiding temperatures between 25 °C and 50 °C will reduce the risk from *Legionella*; however, regulations designed to reduce the risk of scalding can require that hot-water temperatures be kept below 50 °C or even below 45 °C. This can be achieved by lowering water temperatures throughout distribution systems or by installing thermostatic mixing valves close to the point of water use. In either case, greater levels of maintenance will be required to compensate for loss of temperature-based control of *Legionella*.

10.4.1 Managerial responsibilities, registration and notification

Regulations should identify managerial responsibilities associated with systems, and should include requirements for the training and competence of operators. Requirements could also be included for registration of devices with regulatory authorities. Consideration should be given to notification requirements in the event of serious non-compliance.

10.4.2 System assessment and design

System assessment should include inspections of buildings and surrounding areas to identify potential sources of *Legionella* and to evaluate the risk associated with devices, taking into account design, location and operating conditions. A risk assessment could include consideration of:

- the potential for conditions that could favour the survival or growth of *Legionella*
- the potential for production and dissemination of aerosols
- design features, such as deadlegs, the position of air intakes or cooling tower exhausts, and the presence of drift eliminators in cooling towers
- control measures to minimize risks (e.g. automated biocide dosing, flushing, cleaning and general maintenance)

- operating conditions, such as temperature ranges in water distribution systems, and whether devices are operated continuously or intermittently
- the location of devices in relation to exposure of vulnerable groups.

The risk assessment should determine whether existing control measures are sufficient and operate effectively. If they are not sufficient, additional measures should be identified. Guidance on appropriate control measures can be provided in codes of practice referenced within regulations. In addition, regulations can identify specific control measures to be applied, such as:

- application of temperature controls for water distribution systems
- use of biocide dosing as part of an ongoing water management programme
- regular flushing of water systems
- frequencies of cleaning and inspection
- use of drift eliminators on cooling towers.

A number of design features can influence the growth and dissemination of *Legionella*; for example, reducing the occurrence of circulating water temperatures between 25 °C and 50 °C, minimizing stagnant water, installing biocide dosing systems and installing drift eliminators on cooling towers. Cooling towers should be located so that outlets are not close to air intakes or windows of adjacent buildings. Consideration of such features is fairly straightforward when designing new systems, but can present difficulties when dealing with existing systems. The inclusion of design requirements within regulations should be considered. Some existing regulations deal with design, and others do not.

10.4.3 Operational monitoring and verification

Operational monitoring procedures need to be identified for each control measure. Operational monitoring can take the form of testing for defined parameters and inspection programmes. Regulations should include the requirement to institute operational monitoring systems. Operational monitoring requirements regarded as essential could be defined (e.g. the frequency of testing or inspection of cooling towers or water distribution systems). Further guidance on the design and implementation of operational monitoring could be provided in codes of practice and referenced within regulations.

In addition to operational monitoring of individual components and control measures, verification procedures need to be identified. Verification provides reassurance that WSPs as a whole are operating effectively. The process can be undertaken by owner, operators or regulatory authorities, and regulations should specify who is responsible. Verification can include testing for *Legionella*.

Regulations should describe responses when specified requirements are not complied with. Where testing is prescribed, regulations should identify targets and responses to detection. In addition to any immediate remedial action that is deemed necessary, detection of *Legionella* should always lead to a review of risk management procedures. However, failure to detect *Legionella* should not lead to any relaxation in the application of these procedures.

Written procedures for decontamination of devices should be available at the time of commissioning a system, to deal with an outbreak of illness or with other conditions that constitute a substantial risk to public health.

10.4.4 Documentation of management plans and record keeping

All management plans and procedures need to be documented, including those to be followed during normal operation and during incidents and emergencies. The scope and nature of records and documentation should be identified, as should minimum retention times. Requirements for documentation and record keeping should be considered in drafting regulations. Records that could be required include:

- details of building assessments
- plans of water systems
- details of system assessments
- monitoring plans
- results of monitoring, verification, inspections, investigations and any associated remedial action
- the identities of contacts, including managers and/or operators
- results of audits.

10.4.5 Surveillance and audit

Mechanisms for ensuring that appropriate risk management strategies have been implemented should be considered. Similarly, procedures for independent verification and auditing should be considered; these may take the form of regular or random inspections of facilities, devices, documentation and records, and may include testing.

Surveillance agencies should have the authority to enter premises, undertake inspections, review WSPs and results of sampling, and require specific remedial action. Surveillance agencies can include government departments of health, environmental health departments of local government, or agencies with responsibilities for occupational health and safety.

10.4.6 Outbreak investigation and notification of disease

Inclusion of specific regulations to deal with responses to outbreaks should be considered. Such regulations could include provisions for investigations and inspections of devices and documentation by surveillance agencies; they could also include provisions for additional testing and remedial (or even precautionary) decontamination of devices.

A number of countries and regions have established mandatory or voluntary systems for notification of legionellosis, as described in Chapter 9. Such notification can be provided by clinicians or testing laboratories. In Europe, a notification and surveillance scheme has been developed to facilitate detection and investigation of travel-associated infections (EWGLINET, 2003; see Chapter 9).

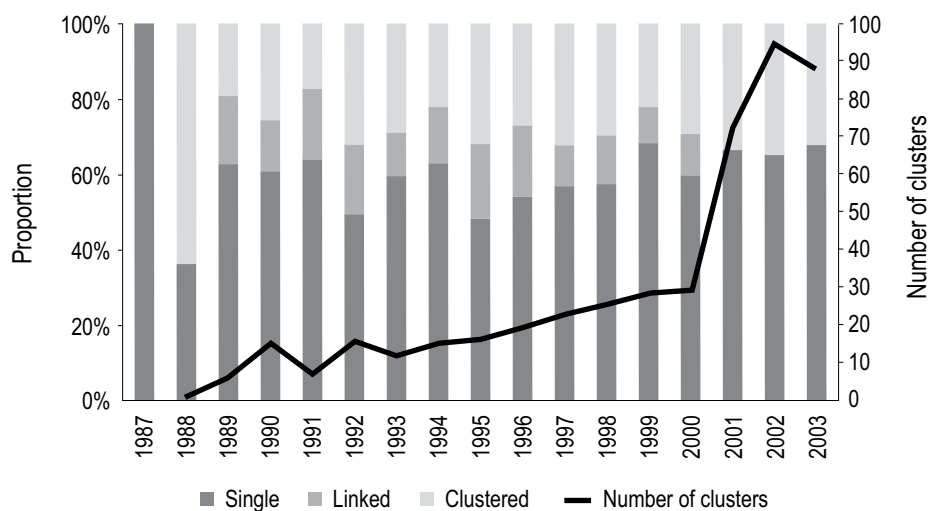
The International Health Regulations (IHR) (WHO, 2005) are a legal instrument designed to provide security against the international spread of infectious diseases. The regulations incorporate provisions for notification and public health responses to events of international significance. Legionellosis is not incorporated in the lists of diseases cited in the IHR (WHO, 2005); however, any disease event that meets the criteria described in Annex 2 of the regulations (*Serious public health impact, unexpected, likely to spread internationally or likely to result in travel or trade restrictions*) must be notified to WHO following the entry into force of the IHR (2005) in June 2007. The IHR (2005) also introduces new requirements for the inspection of ships and the issuing of a ship sanitation certificate which will be relevant to outbreaks associated with ships (see Chapter 7), when these and other provisions in the regulations could be applied.

Notification systems allow prompt investigation of outbreaks or even single cases. Such investigations can lead to the identification of sources of illness, implementation of remedial action and provision of public health advice. As a result, the size of outbreaks and the likelihood of recurrence can be reduced. In some jurisdictions or circumstances, single cases are investigated, on the basis that they may be the first reported case of an outbreak. Investigations of nosocomial cases are considered to be of high importance because of the potential risk to immuno-compromised patients (Lee & Joseph, 2002).

10.5 Outbreak impact and economic consequences

Since the first recognized outbreak of Legionnaires' disease in Philadelphia, USA (Fraser et al., 1977), many outbreaks have been reported, often involving health-care facilities (see Chapter 6). During outbreak investigations and the associated media interest, a more complete picture of the true number of cases is possible than at other times, because during an outbreak clinicians tend to do more diagnostic tests, and reporting of cases is more complete. In Europe, the number of clusters reported to the European Working Group for *Legionella* Infections (EWGLI) is also increasing (see Figure 10.1).

Figure 10.1 Types of *Legionella* cases in Europe, by year of onset



Source: Information obtained from the European Working Group for Legionella Infections (EWGLI)²³

The risk of infection after exposure to *Legionella* is difficult to assess and remains a matter of some debate. Since *Legionella* is ubiquitous in both natural and human-made environments, it must be assumed that most people are exposed frequently, at least to single organisms. Generally, there is either no reaction to such exposure or an asymptomatic production of antibodies. Drinking-water from natural sources and from public supplies may carry single organisms or *Legionella*-containing amoebae. However, other than in health-care facilities, there are no reports of outbreaks or recurrent cases of disease following consumption or use of drinking-water that has been kept cool and not subjected to prolonged periods of stagnation.

Although it is impossible to completely eradicate legionellosis, the risks could be reduced to a tolerable minimum. For example, decontamination of colonized installations has effectively interrupted outbreaks and prevented recurrences of sporadic cases. In two prospective studies conducted in hospitals, the frequency with which *L. pneumophila* was isolated from patients with pneumonia was reduced from 16.3% to 0.1% over a six-year period; similarly, the frequency of isolation from patients who were immunocompromised was reduced from 76% to 0.8% over a 10-year period (Grosserode et al., 1993; Junge-Mathys & Mathys, 1994). These reductions were due to hyperchlorination to prevent nosocomial infections.

Design measures can also help to prevent further outbreaks. For example, after the 1999 outbreak in the Netherlands (Den Boer et al., 2002), the Dutch government launched a plan to combat Legionnaires' disease, emphasizing the need for greater vigilance by general practitioners (GPs) and community health services. The plans included a computerized rapid alert system for GPs, measures to ensure that all GPs and hospital casualty departments are alerted within 24 hours of possible cases of Legionnaires' disease, and stricter controls of public buildings using

²³ <http://www.ewgli.org/>

hot water (e.g. health-care facilities, hotels, saunas and swimming pools). The Dutch College of General Practitioners has also been asked to improve the education of GPs on rare, preventable, infectious diseases. In addition, regulations were drafted and guidance was issued to ensure the safety of water in buildings (see Table 10.1).

Several guidelines for the management of adult community-acquired pneumonia have been published. These include:

- American Thoracic Society guidelines, which were published in 1993 and updated in 2001 (Niederman et al., 1993; ATS, 2001)
- *Guidelines of the Infectious Diseases Society of America*, which were published in 1998 and updated in 2004 (Mandell et al., 2004)
- Canadian guidelines for the initial management of community-acquired pneumonia (Mandell et al., 2002)
- *British Thoracic Society Guidelines* (2001)
- *European Respiratory Society Guidelines* (ESCAP, 1998).

Although these guidelines differ in several treatment recommendations, they uniformly recommend regular antibiotic coverage of *Legionella* spp. in severe pneumonia requiring admission to intensive care units.

Likely benefits of the adoption of the described measures to control and reduce the risks posed by legionellae in cooling tower systems and warm water systems have been discussed in the regulatory impact statement for the Victorian Health (*Legionella*) Regulations (Anon, 2001). The direct benefits are from expected reductions in the incidence of the disease, which would reduce mortality and lead to hospital cost savings. Indirect benefits include savings of medical costs from treating patients, due to an associated reduction in non-fatal incidence of the disease, and a reduction in loss of economic output caused by inability to work. Benefit calculations range from US\$8 million (“worst case”, with 25% effectiveness) to US\$15 million (“best case”, with 50% effectiveness). These calculations do not include any valuation of the estimated 10–20 lives that could be saved over a 10-year period. Given the US\$20–27.5 million range of net present value for the proposed package of controls, including the health regulations for *Legionella*, the implicit costs per life saved range between US\$1 million and US\$3 million (Anon, 2001).

Table 10.1 Selected European regulations developed for the control of *Legionella* in water systems

Country	General prevention						Basis for regulation	Prevention after outbreak	Comments
	DW	Wp	SB	CT	AC	WP			
Austria		x	x				• Health • Bathing hygiene	Yes	Aspects of drinking-water covered by decree of Ministry of Health Special decree for prevention in spa pools and water systems of swimming baths Some provinces: regulations by public health authorities
Belgium (Flanders)	x	x	x	x	x	x	• Environment • Public health • Labour safety • Biosafety	Yes?	Different risk levels covered
Bulgaria	x	x	x		x		• Public health	Yes	
Croatia				x	x	x	• Public health	Yes	Guidelines — Law on communicable diseases
England and Wales	x	x	x	x	x	x	• Health and safety at work • Health • Management of safety at work	?	Primary legislation, approved code of practice and guidance Other legislation: reporting of injuries, diseases; water supply (water fittings); notification of cooling towers

Country	General prevention						Basis for regulation	Prevention after outbreak	Comments
	DW	Wp	SB	CT	AC	WP			
Finland	x		x		x		• Health protection • Housing health • Building code • Communicable diseases	Yes	
France	x (partially)	x		x			• Public health • Drinking-water • Environment	Yes	
Germany	x	x	x	x	x	x	• Public health • Drinking-water • EWGLI	Yes	
Hungary								Yes	There are plans to develop regulations on general prevention of legionellosis
Ireland							• Labour safety	No	Guidelines exist Special attention given to potential risks of dentist systems and high risk in hospitals
Italy	x	x	x	x	x	x	• Public health	Yes	Guidelines for the prevention and control of legionellosis
Latvia	x	x	x	x	x	x	• Labour safety • Public health	Yes	

Country	General prevention						Basis for regulation	Prevention after outbreak	Comments
	DW	Wp	SB	CT	AC	WP			
Lithuania	x (hot water only)						- Public health - Drinking water	Yes (draft)	Recommendations mainly aimed at clinical manifestation, diagnostics and treatment of legionellosis Lithuanian hygiene standard Draft of regulations for legionellosis aimed at prevention in institutions and accommodation where water is stored or used for work
Malta							Public health	Yes	Code of practice for prevention of Legionnaires' disease in hotels and other establishments exists
The Netherlands	x	x	x	x	x	x	- Drinking-water - Bathing hygiene - Safe labour - Infectious diseases - Public health	Yes	Drinking-water decree and guidance document (ISSO publication 55); decree on bathing locations and guidance document; policy rule on working conditions; Public Health Act; Act on infectious diseases.

Country	General prevention						Basis for regulation	Prevention after outbreak	Comments
	DW	Wp	SB	CT	AC	WP			
Poland								Yes	Regulations on <i>Legionella</i> prevention in drinking-water are being discussed Regulation of new buildings construction is being discussed Act on infectious diseases and infections
Portugal									Elaboration of legislation concerning installation and use of air-conditioning and cooling towers equipment Prevention guidelines
Slovenia	x	x	x	x	x		• Environment • Water • Building construction	No	
Sweden	x	x	x	x	x	x	• Public health • Building construction	Yes	Mandatory regulations and general recommendations
Turkey	x	x	x	x	x				

AC = air-conditioning systems; CT = cooling towers; DW = drinking water systems; SB = swimming baths; Wp = spa pools; WP = process water

Source: adapted from International Congress on *Legionella* in Europe: *Problem and Prevention*, 28–29 September 2004, RAI Exhibition and Congress Centre, Amsterdam, The Netherlands²⁴

²⁴ <http://www2.vrom.nl/docs/internationaal/ congres%20questionnaire%20finland.pdf>

Chapter 11 Laboratory aspects of *Legionella*

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This chapter provides:

- background information about *Legionella* biology and staining (Section 11.1)
- information on diagnostic tests for legionellosis (Section 11.2), including:
 - culture (Section 11.2.1)
 - detection of bacterial antigen (Section 11.2.2)
 - detection of bacterial DNA (deoxyribonucleic acid) (Section 11.2.3)
- particular considerations for diagnosing patients with health-care associated (nosocomial) pneumonia (Section 11.2.4)
- approaches to the environmental sampling of *Legionella* (Section 11.3)
- methods for identifying and differentiating *Legionella* species (Section 11.4).

11.1 *Legionella* biology and staining

11.1.1 Biology

Legionella are 0.3–0.9 µm wide and 2–20 µm long, depending on the age of the culture —fresh cultures of *Legionella* produce coccobacilli about 2–6 µm long, whereas older cultures may produce filamentous forms up to 20 µm long. *L. pneumophila* usually has limited motility, and some strains are completely non-motile (Harrison & Taylor, 1988). The bacterium has one or two polar flagellae, the expression of which may depend on temperature (Ott et al., 1991). In contrast to other aquatic bacteria, *L. pneumophila* requires iron salts and the amino acid L-cysteine to grow on laboratory media. Occasionally, rare clinical isolates of three *Legionella* species (*L. jordanis*, *L. oakridgensis* and *L. spiritensis*) may lose their L-cysteine growth dependence (Orrison et al., 1983). This characteristic only develops after serial passage, when *Legionella* from an infected host is used to infect a second host — a process that often results in the mutation of *Legionella* genes not essential for survival. However, legionellae that are not L-cysteine dependent still grow more vigorously on media containing L-cysteine.

11.1.2 Staining

Legionellae are Gram-negative bacteria with a thin cell wall, but stain poorly in the Gram procedure if neutral red or safranin is used as the counterstain. This characteristic is probably due to the composition of legionellae cell walls, which have large amounts of branched-chain cellular fatty acids and ubiquinones with side chains of 9–14 isoprene units (Moss et al., 1977; Lambert & Moss, 1989). Fatty acid and ubiquinone profiling have been used for identifying *Legionella* isolates to the level of species (Benson & Fields, 1998). On its own, Gram staining is inconclusive, even when samples are taken from normally sterile sites, such as transtracheal aspirates, lung biopsies or pleural fluids. Legionellae from these tissues appear as small, Gram-negative rods of varying sizes when counterstained with basic fuchsin. This effect is emphasized in legionellae-infected tissues (Yu, 2000). Dieterle's silver impregnation method is an alternative means of staining legionellae (Dieterle, 1927; Thomason et al., 1979). More sensitive and specific methods of identifying legionellae include antibody-coupled fluorescent dyes and immunoperoxidase staining.

Further information on identifying legionellae species is given in Section 11.4.

11.2 Diagnostic methods

The clinical symptoms of infection with *Legionella* are indistinguishable from the symptoms of other causes of pneumonia. Accurate diagnostic methods are therefore needed to identify *Legionella*, and to provide timely and appropriate therapy. To improve diagnosis, specialized laboratory tests must be carried out, by the clinical microbiology laboratory, on patients in a high-risk category.

Tests for Legionnaires' disease should ideally be performed on all patients with pneumonia at risk, including those who are seriously ill (with or without clinical features of legionellosis), and those for whom no alternative diagnosis prevails. In particular, tests for Legionnaires' disease should be carried out on ill patients who are older than 40 years, immunosuppressed or unresponsive to beta-lactam antibiotics, or who might have been exposed to *Legionella* during an outbreak (Bartlett et al., 1998).

Despite the availability of immunological and molecular genetic methods, diagnosis of Legionnaires' disease is generally effective only for *L. pneumophila* serogroup 1. The sensitivity and specificity of methods for diagnosing and identifying other *L. pneumophila* serogroups and species of *Legionella* are far from perfect (Tartakovsky, 2001).

Since 1995, diagnostic tests for legionellosis have changed significantly. The following laboratory methods are currently used for diagnosing *Legionella* infections (Stout, Rihs & Yu, 2003):

- isolation of the bacterium on culture media
- identification of the bacterium using paired serology
- detection of antigens in urine

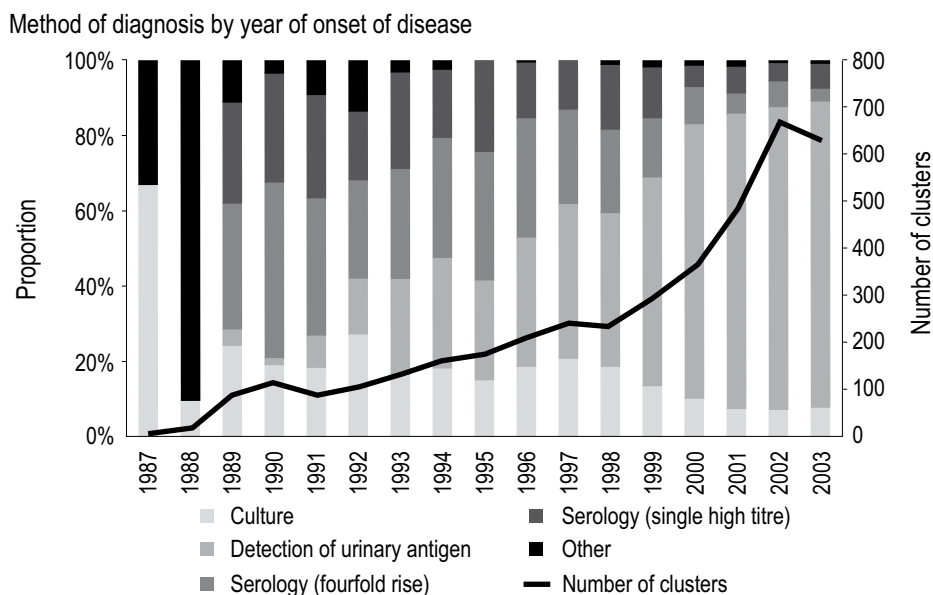
- detection of the bacterium in tissue or body fluids by immunofluorescent microscopy (e.g. direct immunofluorescence assay (DFA) testing)
- detection of bacterial DNA using polymerase chain reaction (PCR).

Table 11.1 compares the sensitivity, specificity and other characteristics of these methods.

Use of culture or DFA techniques has decreased, and most cases of legionellosis are now identified through detection of urinary antigens. As a consequence of this shift, detection of *L. pneumophila* serogroup 1 is increasing, and all other serogroups are probably underdiagnosed.

The highest number of cases of Legionnaires' disease in travellers was reported by the European Surveillance Scheme for Travel Associated Legionnaires' Disease in 1999. This reflects both greater surveillance and an increase in the use of urinary antigen for detecting *L. pneumophila* serogroup 1. Detection of urinary antigen was the most common method of detection (55% of cases; see Figure 11.1). The antigen detection test is substantially more sensitive for community-acquired and travel-associated Legionnaires' disease than for nosocomial (health-care acquired) infection, because the tests are more sensitive for Pontiac *L. pneumophila* serogroup 1 than for non-Pontiac strains; the tests use monoclonal antibodies (MAb) MAb2 or Dresden MAb3/1. Pontiac strains cause the majority of community-acquired and travel-associated Legionnaires' disease cases, but are significantly less common in nosocomially acquired cases.

Figure 11.1 Method of diagnosis of travel-associated Legionnaires' disease in Europe and year of onset of disease



Source: Information obtained from the European Working Group for Legionella Infections (EWGLI)²⁵

²⁵ <http://www.ewgli.org/>

Table 11.1 Comparison of methods for laboratory diagnosis of Legionnaires' disease

Method	Sensitivity (%)	Specificity (%)	Comments	References
Culture			• "Gold standard" • Requires 2–4 days, sometimes (rarely) up to 14 days • Highest specificity	Edelstein & Meyer, 1994; Stout & Yu 1997; Harrison et al., 1998; Maiwald, Helbig & Lück, 1998; Fields, Benson & Besser, 2002; Lück, Helbig & Schuppler, 2002
Sputum	5–70	100		
BAL or transtracheal aspirate	30–90	100		
Lung biopsy	90–99	100		
Blood	10–30	100		
Serology			• Seroconversion may require 3–9 weeks	Edelstein & Meyer, 1994; Plouffe et al., 1995; Stout & Yu, 1997; Harrison et al., 1998; Fields, Benson & Besser, 2002; Lück, Helbig & Schuppler, 2002;
Seroconversion	70–90	95–99		
Single specimen	(unknown)	50–70		
Urinary antigen	75–99	99–100	• Only for L.p.sg1, limited data for other serogroups or species • Very rapid (15 min–3 h), frequently earliest positive finding, may remain positive for several weeks/months	Edelstein & Meyer, 1994; Stout & Yu, 1997; Harrison et al., 1998; Fields, Benson & Besser, 2002; Lück, Helbig & Schuppler, 2002; Uldum & Molbak, 2002
DFA testing			• Very rapid (2–4 h) • Limited sensitivity • Experience needed • No validated reagents for non-pneumophila species	Edelstein & Meyer, 1994; Stout and Yu, 1997; Harrison et al., 1998; Fields, Benson & Besser, 2002
Sputum or BAL	25–75	95–99		
Lung biopsy	80–90	99		

Method	Sensitivity (%)	Specificity (%)	Comments	References
PCR			• Rapid • Diagnostic validity of positive results without confirmation by other methods remains unclear • Detects all <i>Legionella</i> species • Not commercially available	Fields, Benson & Besser, 2002; Lück, Helbig & Schuppler, 2002; Uldum & Molbak, 2002; van der Zee et al., 2002; Roig & Rello, 2003
Respiratory tract specimen	85–92	94–99		
Urine, serum	33–70	98–98		

BAL = bronchoalveolar lavage; DFA = direct immunofluorescence assay; L.p.sg1 = *Legionella pneumophila* serogroup 1; PCR = polymerase chain reaction

11.2.1 Diagnosing legionellosis using culture media

Before the development of an in vitro medium that could sustain legionellae (Feeley et al., 1978; Feeley et al., 1979), legionellae could only be grown by isolating them in guinea pigs or hen eggs (McDade et al., 1977; Morris et al., 1979). Currently, the preferred technique for checking other diagnostic methods is to grow the bacteria on direct culture.

Primary isolation of *Legionella* spp. is carried out using a defined *Legionella* agar medium containing L-cysteine, such as buffered charcoal yeast extract (BCYE) agar. Supplements that reduce the background competing bacterial flora and yeasts may be added to increase selectivity of the media. These supplements include BCYE-agar with anisomycin (Dournon, 1988), BMPA α medium with buffered cefamandole, polymixin B, or anisomycin agar (Edelstein, 1981). It is best to use both selective and nonselective agars, because cefamandole may inhibit some *Legionella* species (Edelstein, 1981). Supplemented BCYE medium is the most commonly used. This medium can be easily prepared by any large clinical microbiological laboratory and can be made in a semiselective form. However, supplements need to be added carefully so that they are not overheated. The quality of each batch of the media (i.e. each flask) must be checked using *Legionella* strains that have not been adapted to laboratory media by successive subculture. This is because laboratory strains adapt to laboratory media and are less sensitive to poor-quality media than fresh isolates of *Legionella* from clinical and environmental samples.

Culture yield is greatest in highly experienced laboratories using multiple media and pre-plating specimen decontamination. Culture plates are incubated at 36 $\pm$ 1 °C for up to 14 days and are examined every two or three days. Even the detection of one or a few colonies is sufficient to confirm the diagnosis. The appearance of colonies may be delayed if patients have received appropriate antibiotics, and if the specimen is contaminated with other microorganisms or another species (Stout & Yu, 1997; Lück, Helbig & Schuppler, 2002).

Ideally, specimens for culture should be taken before antibiotic treatment is initiated, although *Legionella* has been isolated from lower respiratory tract specimens and blood after several days treatment with erythromycin. Sputum should be considered for culture even when not purulent (Ingram & Plouffe, 1994). Respiratory specimens that are particularly difficult to obtain, such as lung tissue, pleural fluid or bronchoalveolar lavage (BAL), should be cultured if received on a routine basis (Stout, Rihs & Yu, 2003).

Legionella has been successfully isolated from lower respiratory tract specimens, including BAL, transtracheal aspirate, endotracheal suction specimens, pleural fluid, lung biopsy and expectorated sputum. In the early phase of illness, legionellosis is often accompanied by a dry cough with little sputum. In this context, the low number of organisms present outside the lungs and the inhibitory effect of oral flora reduce the sensitivity of the culture method. In severe forms of legionellosis, especially in immunocompromised patients, bacteraemia (bacterial spread to the bloodstream) can occur, with a frequency of approximately 30% in patients with severe legionellosis. Sometimes, legionellae are found in samples from extrapulmonary sites, especially from postmortem specimens (e.g. liver, spleen, pericardial fluid, kidney wounds, cutaneous abscess or vascular grafts).

Benefits and limitations of using culture media

Culture of *Legionella* is often the most sensitive detection method, and has high specificity (>99%) (Edelstein, 1987). Culture is particularly important for diagnosis in:

- cases in which severe pneumonia causes respiratory failure
- immunocompromised patients
- nosocomial infections
- cases in which disease is caused by any legionellae other than *L. pneumophila* serogroup 1.

Some legionellae cannot be grown on routine *Legionella* culture media and have been termed *Legionella*-like amoebal pathogens (LLAPs), because they grow in certain host species of amoeba. These organisms have been isolated and maintained in culture by co-cultivating the bacteria with their protozoan hosts. One LLAP strain was isolated from the sputum of a pneumonia patient by enrichment in amoebae. This LLAP strain is considered to be a human pathogen (Fry et al., 1999; Marrie et al., 2001). Other LLAP strains may be human pathogens, although this is difficult to prove because they cannot be detected by conventional techniques used for legionellae. Recently, three LLAP strains were named *Legionella* species (Adeleke et al., 2001; La Scola et al., 2004).

11.2.2 Detecting *Legionella* antigens

Urinary antigens

Enzyme immunoassays

The use of enzyme immunoassays (EIAs) for detecting *L. pneumophila* antigen in urine allows Legionnaires' disease to be diagnosed early in the course of infection. EIA is a convenient and rapid test with excellent specificity and sensitivity for *L. pneumophila* serogroup 1. The antigen is detectable in most patients between one and three days after the onset of symptoms, and may persist for some weeks or months — even when other tests can no longer detect the antigen (Birtles, 1990). The EIA urine antigen test has 80–85% specificity, which is similar to culture (Hackman et al., 1996; Kazandjian, Chiew & Gilbert, 1997), but may have greater sensitivity than culture. Commercial EIA kits are available for detecting *L. pneumophila* serogroup 1 antigen in urine.

Immunoassay for detection of urinary antigen is the method of choice for *L. pneumophila* serogroup 1 (Cosentini et al., 2001; Formica et al., 2001). Compared with other diagnostic methods, the advantages of urinary antigen detection are striking. Specimens are easily obtained, the antigen is detectable very early in the course of disease, and the test is rapid and specific. The antigen might also be detectable in non-pneumonic illnesses and during antibiotic therapy (Lück, Helbig & Schuppler, 2002).

Immunochromatographic assays

A rapid immunochromatographic assay for detecting *L. pneumophila* serogroup 1 antigen in urine is also available. This assay detects urinary antigen within a very short time and does not require laboratory equipment (Helbig et al., 2001). Concentration of urine improves the sensitivity of both the EIA and immunochromatographic assays, without decreasing their specificity.

Tissue antigens

Indirect immunofluorescence microscopy

Immunofluorescence microscopy can be used to detect *Legionella*, using either direct or indirect techniques, in samples such as respiratory tract secretions, lung and pleural fluid. The indirect immunofluorescence antibody technique (IFAT) is used in most laboratories to detect the serum antibody level. A fourfold rise in titre develops within 1–9 weeks after disease onset in approximately three quarters of patients with culture-proven legionellosis caused by *L. pneumophila* serogroup 1. On average, patients seroconvert (develop antibodies) within two weeks; however, up to 25% of seroconversions are undetected because serum is collected more than eight weeks after disease onset.

In the clinical setting, serology is limited in its usefulness as a diagnostic tool for legionellosis because of the length of time required, the need for paired sera, and the difficulty of obtaining appropriate convalescent samples (Stout & Yu, 1997). Although diagnosis by antibody detection from tissues is still useful for epidemiological studies in outbreaks or to establish an infection retrospectively, it has generally been superseded by the urinary antigen test, as discussed above. A single high titre with clinical symptoms suggestive of legionellosis gives a presumptive diagnosis. However, in one study, a single acute-phase antibody titre of 1:256 could not discriminate between cases of *Legionella* and non-cases (Plouffe et al., 1995). Cross-reactions with other bacteria, such as *Campylobacter* and *Pseudomonas* species, have also occurred (Marshall, Boswell & Kudesia, 1994; Boswell, Marshall & Kudesia, 1996; Harrison, 1997).

Indirect IFAT is used to diagnose legionellosis by incubating samples with a hyperimmune antiserum and then visualizing them by applying a fluorescently tagged anti-*Legionella* antibody, fluorescein–isothiocyanate-conjugated immunoglobulin (FITC). A positive control (human reference serum) and a negative control (human serum from a healthy individual) are required (Rose et al., 2002). The sensitivity and specificity of IFAT have only been evaluated using *L. pneumophila* serogroup 1 antigen; sensitivity and specificity for other serogroups or species are not known (Muder 2000; Lück, Helbig & Schuppler, 2002). Because of the formation of cross-reactive antibodies, about 50% of patients infected by *L. pneumophila* non-serogroup 1 seroconvert with antigens specific to *L. pneumophila* serogroup 1 (Edelstein, 2002). A negative result does not exclude legionellosis, and care needs to be taken to confirm a positive result when low numbers of bacteria are seen (Benson & Ward, 1992).

Antigen preparation differs between laboratories and manufacturers, resulting in different critical titre levels. For some antigen preparations, specificity could be relatively high for a single specimen, and low for another antigen (Rose et al., 2002).

A number of companies produce FITC-labelled antibodies for the detection of *L. pneumophila*. An FITC-conjugated monoclonal antibody (MAb) directed against *L. pneumophila* common outer-membrane protein is commercially available, and is preferred because it is more specific than polyclonal reagents. The MAb has the advantage of reacting with all *L. pneumophila* serogroups, but only identifying *L. pneumophila*. Genus-specific MAbs are not suitable for immunofluorescence.

Direct immunofluorescence assays

Direct immunofluorescence assays (DFAs) using antibody conjugated with a fluorochrome require 2–3 hours to complete the staining procedure. DFAs for *Legionella* species other than *L. pneumophila* should not ordinarily be used. DFA of sputum remains positive for 2–4 days after the initiation of the specific legionellosis antibiotic therapy, and often for a longer period in cases of a cavitary pulmonary disease (Lück, Helbig & Schuppler, 2002).

DFA has been used successfully with expectorated sputum, endotracheal suction aspirates, lung biopsies and transtracheal aspirate (Stout, Rihs & Yu, 2003). Pleural fluid examination in patients with legionellosis by culture or DFA rarely yields positive results, but has occasionally been helpful. Between 25% and 70% of patients with culture-proven legionellosis have positive DFA for *L. pneumophila*, and the test's specificity is higher than 99.9%. Therefore, a negative result does not rule out legionellosis but a positive result is almost always diagnostic, provided that the slide is read correctly.

Care must be taken to prevent false-positive results of DFA. These can result from clinical specimens coming into contact with contaminated water, such as contaminated buffers or organisms washed from positive control slides (Lück, Helbig & Schuppler, 2002). In addition, skill and experience are required to interpret the DFA; therefore, laboratories lacking expertise should be discouraged from using it.

Enzyme immunoassays

Microagglutination or enzyme immunoassay (EIA) methods can be used to serologically diagnose *L. pneumophila* serogroup 1 in tissues (Edelstein, 2002). Several EIA serologic diagnostic kits are commercially available, with sensitivity ranging from 80% to 90% and a specificity of about 98%. The sensitivity of kits for testing antibody from serotypes 2–6 is still unknown. The conformity of EIA tests with the immunofluorescence method is about 91% (Edelstein, 2002).

11.2.3 Diagnosing legionellosis using nucleic acid detection

Overview of polymerase chain reaction assays

L. pneumophila DNA was first detected in clinical samples by a commercial nucleic acid hybridization assay that used a radioisotopically labelled RNA (ribonucleic acid) probe. However, concerns about the sensitivity and specificity of the assay led to its subsequent withdrawal (Fields, Benson & Besser, 2002).

Since then, *Legionella* polymerase chain reaction (PCR) assays have been used more actively to detect DNA from environmental samples, but can also be used for analysing clinical samples, particularly those from the respiratory tract. Detection of *Legionella* and *L. pneumophila* DNA has been reported using PCR assays (with or without confirmation by blot hybridization or sequencing) (Mahbubani et al., 1990; Lisby & Dessau, 1994; Ko et al., 2003; Liu et al., 2003), including those targeting:

- ribosomal RNA (rRNA) genes or their intergenic spacer regions
- a gene coding for heat-shock protein (*dnaJ*)
- the RNA polymerase gene (*rpoB*)
- the macrophage infectivity potentiator (*mip*) gene.

Traditionally, the rRNA genes have been used for assays targeting the *Legionella* genus and the *mip* gene for *L. pneumophila*-specific assays. Assays for *Legionella* and *L. pneumophila* using direct (real-time) monitoring platforms have also been described (Ballard et al., 2000; Hayden et al., 2001; Rantakokko-Jalava & Jalava, 2001; Wellinghausen, Frost & Marre, 2001). With respiratory samples, *Legionella* PCR has a reported specificity of $\geq 99\%$ and sensitivity of 85% (Edelstein & Meyer, 1994; Fields, Benson & Besser, 2002; Lück, Helbig & Schuppler, 2002; Uldum & Molbak, 2002).

An important feature of *Legionella* PCR is that the method can potentially detect all serogroups of *L. pneumophila* and is therefore useful in the early diagnosis of infections, particularly in nosocomial cases (Uldum & Molbak, 2002). Over the past few years, PCR techniques have improved substantially, particularly those for direct (real-time) monitoring of the generation of PCR fragments. The use of real-time PCR technique accelerates the diagnostic procedure for legionellosis and improves the specificity.

PCR methods could have important economic benefits. Their use in outbreaks of legionellosis could help to rapidly rule out implicated sites, thereby minimizing lost revenue and allowing resources to be diverted to areas that need further investigation. Until the diversity and distribution of legionellae are better understood, results from methods other than culture should be interpreted cautiously.

Limitations of PCR assays

Current data are insufficient for reliably estimating PCR sensitivity and specificity values, or for comparing PCR to other methods. Broadening the application of PCR requires evaluation and standardization of sample preparation and PCR protocols, to define primer and probe specifications and assay sensitivities, and to reduce the effect of PCR inhibitors. Ideally, internal process controls should also be included, to indicate the presence or absence of PCR inhibitors or the failure of PCR (Ursi et al., 1992; Hu et al., 2002; Lück, Helbig & Schuppler, 2002).

Few validation data are available for the many assays described, particularly for use in a clinical setting. *L. pneumophila* PCR assays appear to be promising, but assays reported to specifically target all *Legionella* species should be viewed with caution and carefully assessed. Before a specific PCR assay is used to diagnose legionellosis, its analytical sensitivity and specificity should be determined and compared with that of other PCR assays.

11.2.4 Diagnosing patients with health-care associated pneumonia

Nosocomial Legionnaires' disease is discussed in detail in Chapter 6. This section applies to diagnostic testing used to evaluate patients with health-care associated pneumonia.

Diagnosing patients with nosocomial pneumonia requires the following:

- Every health-care facility should have access to a laboratory that is proficient in isolating *Legionella* from cultures and has urine antigen testing facilities.

- Serologic testing can be used for diagnosis, but is not the most helpful diagnostic tool, because a fourfold rise in antibody titre from specimens obtained 3–6 weeks apart is necessary to make a clinical diagnosis of legionellosis; a diagnosis is rarely made from a single high titre.
- DFA testing can be used for diagnosis; however, testing using this method must be regular so that changes in results can be detected immediately.

The consequences of failing to regularly test patients with health-care acquired pneumonia were identified in a study by Lepine et al. (1998), who reported a cluster of cases of legionellosis in a hospital soon after the introduction of urine antigen testing. The hospital had experienced an outbreak of nosocomial legionellosis 16 years earlier and, as revealed by molecular subtyping methods, the isolates from the two outbreaks were identical. There was no increase in the hospital's overall rate of nosocomial pneumonia. The study suggested that persistent transmission of *Legionella* infections may have been occurring over a long period, without being recognized.

Several investigations report underuse of diagnostic testing. Fiore et al. (1999) published a survey sponsored by the Centers for Disease Control on surveillance systems for health-care acquired infections. Of the 192 hospitals that responded, only 60% could provide in-house testing for legionellosis, and only 21% had established routine testing procedures that included legionellosis for respiratory specimens from patients with nosocomial pneumonia. This study highlights the importance of surveillance for legionellosis and infection control in hospitals, residential institutions and other such buildings.

Health-care facilities must have policies in place to test for legionellosis in patients with nosocomial pneumonia. Effective diagnosis and evaluation of results are crucial for the adequate and prompt management of incidents and outbreaks, for the control of clusters of infections, and for the protection of other patients.

11.3 Analysing environmental samples for *Legionella*

11.3.1 Standards for *Legionella* detection and recovery

There are a number of manuals and laboratory procedures for the recovery of legionellae from environmental samples. In 1998, an international standard (International Organization for Standardization ISO 11731) was developed to incorporate the different strategies used by a number of institutions for efficient recovery and detection of legionellae (ISO, 2004). The following sections provide an overview of methods for detecting the bacterium in water samples, according to the ISO standard.

11.3.2 Ensuring safety during environmental sampling

Environmental samples of *Legionella* should be collected by people with knowledge of *Legionella* ecology and general risk assessment (see Chapters 2 and 9). People taking environmental samples require training to ensure that they select samples containing the highest numbers of

bacteria, and that they are aware of the risk to themselves and to others from potentially positive sites. In some circumstances, it may be necessary to use respiratory protective equipment, although in most cases cooling systems can be turned off to allow safe sample collection. An exception may be where a wet cooling system is being used to cool a potentially explosive industrial process. In that situation, a risk assessment must be made before sampling. If it is not safe to take samples, the system should be rendered safe as soon as possible.

Several countries or regions produce guidelines on sampling. Advice on methods that comply with the European and United Kingdom guidelines has recently been published and is freely available from the Internet (Standing Committee of Analysts, 2005).²⁶

11.4 *Legionella* speciation and serology typing

11.4.1 Identifying different *Legionella* species

Methods used to identify and differentiate *Legionella* species include (Benson & Fields, 1998; Ratcliff et al., 2003):

- phenotypic characteristics
- growth requirements
- biochemical characteristics
- fatty acid and carbohydrate analysis
- ubiquinones
- protein profiling
- serology
- monoclonal antibodies reactions
- various molecular techniques (including, recently, the use of sequencing techniques).

The use of biochemical profiles for routine identification of legionellae other than *L. pneumophila* is limited. Legionellae test positive for catalase — an enzyme in blood and cells that catalyses the decomposition of hydrogen peroxide into oxygen and water. The oxidase reaction gives variable results and is therefore not very useful. Reactions for nitrate reduction, urease and carbohydrate use are negative. Most species of *Legionella* produce beta-lactamase, lipase and phosphatase (Thorpe & Miller, 1981) and liquefy gelatine. Strains belonging to all serogroups of *L. pneumophila*, except serogroups 4 and 15, strongly hydrolyse hippurate (Hebert, 1981). Several laboratories have described methods for identifying putative *Legionella* isolates to the genus level, and in some cases to the species level, using only phenotypic characteristics.

²⁶ http://www.environment-agency.gov.uk/commercial/1075004/399393/401849/?version=1&lang=_e

Although not all strains can be reliably identified to the species level, narrowing strains to groups is useful, and this is usually achieved using serology. DNA–DNA hybridization best identifies a strain of *Legionella* or a new species. The procedure requires DNA from the test strain to be hybridized with DNA from all known species of *Legionella*, and is therefore only undertaken by specialized laboratories. Sequence analysis of specific genes has been used for taxonomic analysis of legionellae. Analysis of 16S rRNA genes led to the designation of *Legionella* within the gamma-2 subdivision of the class Proteobacteria, and has been used to show the phylogenetic relatedness of new species of this genus (Fry et al., 1991). A sequence-based classification scheme that targets the *mip* gene has been developed for legionellae (Ratcliff et al., 1998). This scheme can unambiguously discriminate between the 39 species of *Legionella* tested so far, and it is likely that all taxonomic analysis will soon become sequence-based.

Within the genus *Legionella*, species can therefore be distinguished by biochemical analysis, fatty acid profiles, protein banding patterns, serology, DNA–DNA hybridization and analysis of 16S rRNA genes (Hookey et al., 1996; Benson & Fields, 1998; Riffard et al., 1998; Fields, Benson & Besser, 2002).

11.4.2 Identifying *Legionella* colonies

Steps for identifying and confirming *Legionella* colonies are the same, irrespective of whether the isolates are from clinical or environmental samples. Young, presumptive colonies of *L. pneumophila* show a characteristic speckled green, blue or pink–purple iridescence. More mature colonies (after three or four days) have entire margins, and are convex, 3–4 mm in diameter and like frosted glass in appearance. Older colonies lose most of their iridescence. Subsequent confirmation should be carried out using a cysteine-free agar to show dependency on L-cysteine (Barker, Farrell & Hutchinson, 1986).

The rapid identification and separate confirmation of *L. pneumophila* serogroup 1, other serogroups and some other pathogenic species is important for epidemiological investigations. Presumptive colonies of pathogenic *Legionella* species from clinical or environmental samples can be confirmed using a range of antibody reactions, such as indirect immunofluorescence, direct immunofluorescence, immunodiffusion, crossed immunoelectrophoresis and slide agglutination.

Preliminary identification of *Legionella* spp. with an antibody-reaction test can be done by routine microbiological laboratories. Commercially available latex agglutination kits may be used for confirmation. Suspect colonies are simply emulsified as directed, and mixed with each latex reagent separately on a disposable reaction card. Each reagent is sensitized with antibodies specific to *Legionella*. In the presence of homologous antigens, the latex particles agglutinate to give a clearly visible positive reaction for some minutes (Hart et al., 2000). Isolates that react with specific antisera against known legionellae are confirmed legionellae. The different serogroups of *L. pneumophila* may cross-react (Wilkinson et al., 1990), and when isolates fail to react with specific antisera to all known legionellae, they must be evaluated and eventually

considered as potential new species. A more detailed identification can be carried out in reference laboratories.

Both environmental strains and clinical isolates can be successfully subtyped by molecular techniques, such as ribotyping, macrorestriction analysis by pulsed-field gel electrophoresis (PFGE), or PCR-based methods (Schoonmaker, Heimberger & Birkhead, 1992; Pruckler et al., 1995; Van Belkum et al., 1996; Ballard et al., 2000). The most accurate way to compare epidemiologically linked environmental and clinical isolates is to use two typing methods — genotyping (e.g. PFGE) and phenotyping (monoclonal subtyping) — in parallel (Drenning et al., 2001). An internationally recognized typing method using amplified fragment length polymorphism (AFLP) was tested on *Legionella* infections by 11 countries within the European working group (Fry et al., 2000; 2002). This method allows culture media to be compared without the need for transportation. In future, sequence-based typing methods, such as that described by Gaia et al. (2003), will be more commonly used. A consensus sequence-based scheme based on this previous work, using a standard protocol and dedicated web site²⁷ will greatly assist in timely epidemiological investigation, particularly of travel-associated cases of infection caused by *L. pneumophila* (Gaia et al., 2005).

11.4.3 Identifying appropriate sampling sites

Selection of sampling sites depends on whether the sampling is for routine monitoring or to investigate an outbreak. The use of PCR for detecting nucleic acids of legionellae in the environment has been valuable in some investigations of legionellosis outbreaks, and is particularly useful for eliminating epidemiologically and geographically implicated sources. Quantitative methods are being developed for determining whether a potential environmental source is above guideline or mandatory levels contained in national legislation, where available (Ballard et al., 2000). The use of PCR to detect legionellae in the environment shows that up to 80% of fresh water is positive; this compares with only 20–40% when using culture to detect *Legionella*. The discrepancy could be due to the presence of non-viable or injured organisms, viable but non-culturable legionellae, a nonspecific reaction with unrelated organisms (although data suggest this is not the case), or the presence of new species of legionellae.

The number and types of sites that should be tested to detect legionellae must be determined on an individual system basis. This is because of the diversity of plumbing, heating, ventilation and air-conditioning systems in the various institutions that may be sampled, which include industrial facilities, hotels, hospitals, retirement homes, public facilities and domestic environments. In 1987, an environmental sampling protocol was published, dealing with selection of appropriate sites to sample within a hospital (Barbaree et al., 1987) (see Table 11.2). This protocol can serve as a prototype for identifying sites that should be sampled in various institutions.

²⁷ <http://www.cwgli.org>

Generally, any water source that may produce aerosols should be considered a potential source for the transmission of legionellae (see Chapter 2). Legionellae require temperatures between 20 °C and 50 °C to multiply. Consequently, the bacteria are rarely found in municipal water supplies and tend to colonize warm water systems and point-of-use devices, particularly hot-water systems. Legionellae do not survive drying, and so condensation from air-conditioning equipment, which frequently evaporates to dryness, is not a likely source. High numbers of environmental legionellae, which grow only at 30 °C or below and will not grow at 37 °C, have been isolated from cold systems (Vladimir Drasar, OHS National Legionella Reference Laboratory, Czech Republic, personal communication, January 2005); however, these are unlikely to have any clinical significance.

Table 11.2 Examples of environmental sites for sampling for legionellae

Site	Approximate number of samples	Volume of samples
Potable water outside or on boundary of health-care facility property		
Treatment plant (raw and refined water)	2	10 litres
Guardhouse or other facility if water is not fed from health-care facility	1	1 litre
Fire hydrants	2	1 litre
General potable water system for health-care facility		
Incoming water pipe(s)	2	10 litres
Water softener (pre and post)	2	1 litre
Preheater (discharge side)	1	1 litre
Primary heater (discharge side)	1	1 litre
Circulating pumps	2	1 litre
Holding tanks (cold water, discharge side)	2	1 litre
Expansion tank for hot water	1	1 litre
Back drain on sprinkler system(s)	2	1 litre
Fireline where it branches off main system	1	1 litre
Water used for respiratory therapy equipment	2	≥ 1
Outlets in patients' rooms	4	1 litre
Air compressor system		
Vacuum water source	1	≥ 100 ml
<i>Positive pressure equipment side</i>		
Condensate from tank(s)	3	≥ 100 ml
Water separator(s) directly off compressors	4	≥ 100 ml

Site	Approximate number of samples	Volume of samples
Water source(s) near air intake(s)	4	≥ 100 ml
Air samples where patients were ill with legionellosis	3	n.a.
Potable water final distribution outlets		
<i>Haemodialysis water source</i>		
Before or after demineralizer	1	≥ 1 litre
Intensive care units		
Respiratory therapy (patients' room)	2	1 litre
Cardiac	2	1 litre
Services with different geographical locations	7	1 litre
Ice-maker (entry water) and ice		≥ 1 litre
Air-conditioning system		
Air handling unit serving area where disease occurred	2	≥ 100 ml
<i>Cooling towers</i>		
Return from heat exchanger to water (spray/trough and gutter) distribution or pond (sump)	2	≥ 1 litre
Water supply	1	1 litre
Hot tubs		
Pool and balance tank (if fitted)	1	1 litre
Jets and pipes	1	Swab
Other		
Decorative fountain	1	1 litre
Creeks, ponds, sites of stagnant water	4	>1 litre

n.a. = not applicable

Source: Adapted from Barbaree et al., 1987

11.4.4 Collecting environmental samples

Two primary sample types — water samples and swabs of point-of-use devices or system surfaces — should be collected when sampling for legionellae. Collection of at least 1 litre of water allows the sample to be concentrated, if necessary. If the water source has recently been treated with an oxidizing biocide, such as chlorine or bromine, sodium thiosulfate must be added to each 1-litre sample in sufficient quantities to neutralize any disinfectant present. Depending on the reason for sampling, the sample may be taken as a first flush (i.e. no disinfection). This is appropriate for most occasions and will represent the worst case. After disinfection, the sample will be taken from a running outlet representing the circulating system.

During outbreak investigations, swabs should be taken in conjunction with water samples from sites where biofilms are likely to form. These swabs can be taken from various points within plumbing systems, from surfaces such as biofilms, and from areas that are difficult to reach, such as within the jets of hot tubs (see Chapter 8), thermostatic mixer valves or showers. The swabs can be submerged in a small volume of water taken at the same time, or in Pages's saline to prevent drying during transportation to the laboratory.

All samples should be transported to the laboratory in dark, insulated containers to protect them from extreme temperatures and from light.

Information should be gathered to help interpret the results. As a minimum, the following information should be included on the request form:

- the site and sample point
- the sample references and date
- the reason for sampling
- the temperature of the sample source (e.g. the temperature of a hot-water system at one minute after turning on the tap, and at two minutes after turning on the cold tap)
- any biocide used
- the timing of the dosage in relation to sampling
- the concentration detected at the time of sampling
- any other risk factors of importance (e.g. closed system opened for maintenance)
- high risk of nutrient present, such as in plastics manufacturing plants
- any cases associated with the site.

11.4.5 Sample preparation and isolation

Isolation methods for clinical and environmental samples differ. Legionellae are usually a very minor component of the total bacterial population in environmental samples, and are rarely present in high numbers. Thus, when working with environmental samples, it is usually necessary to first concentrate the microfloras. In the case of clinical specimens such as sputa and tissue biopsies, these may need to be homogenised before culture; in contrast, the organisms in fluids such as bronchiolar lavages will need to be concentrated by centrifuging. For both environmental concentrates and clinical samples, it is necessary to eliminate or suppress the competing background flora during primary culture.

Legionellae and background bacteria can be concentrated from water samples by centrifugation or membrane filtration, or by a combination of the two. Recovery in the presence of other bacterial species present in the sample can be improved by heating, usually at 50 °C for

30 minutes (Maiwald, Helbig & Lück, 1998), and by treating with acid (Bopp et al., 1981; ISO, 1998; ISO 2004). If using an acid treatment, an acid buffer of pH 2.2 should be used for five minutes, although this may also inhibit the growth of legionellae (Lück, Helbig & Schuppler, 2002). Homogenates of sputa and tissues, and centrifuged deposits from more fluid clinical specimens should be cultured directly, and after treating with heat or an acid buffer (Stout, Rihs & Yu, 2003).

Although it may be possible to isolate legionellae on the non-selective growth medium BCYE (particularly from clinical specimens), it is usually necessary to use modified versions of BCYE containing an antibiotic supplement to suppress the background flora, such as:

- polymixin, anisomycin and cefamandole (Edelstein, 1981)
- glycine, vancomycin and polymixin, plus one of the following:
 - cycloheximide (Dennis, 1988b)
 - natamycin, which is an alternative antifungal to cycloheximide that is less toxic to humans (Edelstein & Edelstein, 1996).

An alternative medium — Wadowsky and Yee medium (MWY) as modified by Edelstein (1982b) — includes vancomycin, bromothymol blue and bromocresol purple, and is used to increase the differentiation of legionellae from the background organisms (Wadowsky & Yee, 1981; Vickers et al., 1981). Morrill et al. (1990) advocated the additional use of albumin to increase the recovery of *L. micdadei* and *L. bozemanii*.

In environmental investigations of outbreaks of legionellosis, culture has been used to detect legionellae in the environment. As a result, most of the epidemiologically relevant information concerning legionellosis is based on direct culture data. All agar plates are inoculated with a portion of sample (generally 0.1–0.2 ml) by the spread plate technique and incubated at 36 °C, preferably in a humidified 2.5% carbon dioxide (CO₂) atmosphere or candle extinction jar.

11.4.6 Interpreting results

To date, no direct relationship has been established between the risk of infection and the number of *Legionella* detected in a water system using the generally adopted culture method. Recovery of *L. pneumophila* by culture is poor because:

- *Legionella* exist with other background heterotrophic bacteria; therefore, the sample needs to be treated with heat or acid to repress the growth of non-*Legionella* bacteria on the culture media
- antibiotics need to be added to the culture medium for *Legionella* growth.

- other *Legionella* species that do not cause legionellosis produce colonies on the medium, as does *L. pneumophila*
- the culture technique often fails to detect some other disease-causing *Legionella* species (e.g. *L. bozemanii* and *L. micdadei*)
- residual disinfectant in the system may affect the cultivation of legionellae
- if the sample collection bottles do not contain a neutralizing agent, *Legionella* may be killed (Wiedenmann, Langhammer & Botzenhart, 2001).

These uncertainties and differences in susceptibility of *Legionella* populations make it difficult to interpret the colony count values for *Legionella* in relation to disease risk. However, culture results, together with the percentage of samples containing *Legionella*, provide useful information about the degree of amplification of *Legionella* in a system. A high degree of amplification results in a higher exposure, which may be related to a higher infection risk.

When using L-cysteine dependence to confirm legionellae, it is worth remembering that some bacteria produce extracellular cysteine that can support the growth of legionellae, which then appear as satellite colonies on the cysteine-free medium (Wadowsky & Yee, 1983). Some pseudomonads can grow with *Legionella* within water systems; however, their presence can reduce *Legionella* growth on artificial media. When this occurs and the pseudomonads cannot be removed by pretreatment or dilution, the results must be interpreted carefully.

Appendix 1 Example of a water system checklist

Tasks to be done:

Date:

Task	Yes	No	Observations
Check the water tank and the water tank chlorinator			
Measure chlorine in water in tank			Measurement: ppm
Measure chlorine in the municipal water supply			Measurement: ppm
Measure temperature of the water in the tank			Measurement: °C
Raise the hot water in the taps to 70°C for 2 hours			
Purge the fire-fighting water system			
Check storage tanks and thermometers in the boiler room			
Check air-conditioner and heat pump filters			
Clean and disinfect ornamental fountains			
Present the weekly record sheets to the hotel manager			
Update the plans of the installation			
Observations:			

Signature of technician responsible:

Signature of hotel manager:

Monthly check of hot/cold water temperatures in guestrooms

Ensure that the temperature of the hot and cold-water taps and showers in all guest rooms in the hotel is checked once a year, by spreading the total number of guest rooms over the 12 months of the year.

Check a range of taps and showers, including some that are close to, and some far from, the hot water storage system and the water tanks, and some that are on different floors.

Inspect the shower heads and the filters on the taps, so they can be cleaned where necessary.

Month:

Hot water				Cold water		
Room number	Tap	Shower	Check shower heads	Tap	Shower	Check shower heads
	These temperatures should be above 50°C			These temperatures should be below 20°C		

Weekly water system check list

Week:

Monday			Friday		
Chlorine cold water; room number			Chlorine of cold water; room number		
Temperature of hot water; room number			Temperature of hot water; room number		
Chlorine of cold water; room number			Chlorine of cold water; room number		
Temperature of hot water; room number			Temperature of hot water; room number		
Temperature; boiler number			Temperature; boiler number		
Tuesday			Saturday		
Chlorine cold water; room number			Chlorine of cold water; room number		
Temperature of hot water; room number			Temperature of hot water; room number		
Chlorine of cold water; room number			Chlorine of cold water; room number		
Temperature of hot water; room number			Temperature of hot water; room number		
Temperature; boiler number			Temperature; boiler number		
Purge hot water storage tanks	Y	N	Bleed taps in unoccupied rooms	Y	N
Wednesday			Sunday		
Chlorine cold water; room number			Chlorine of cold water; room number		
Temperature of hot water; room number			Temperature of hot water; room number		
Chlorine of cold water; room number			Chlorine of cold water; room number		
Temperature of hot water; room number			Temperature of hot water; room number		
Temperature; boiler number			Temperature; boiler number		
Thursday					
Chlorine of cold water; room number					
Temperature of hot water; room number					
Chlorine of cold water; room number					
Temperature of hot water; room number					
Temperature; boiler number					
Observations:					

Signature of technician responsible:

Signature of hotel manager:

Appendix 2 Example of a 2-week follow-up form

This appendix provides an example from the United Kingdom of a form for local use, for collecting a 2-week history before onset of Legionnaires' disease.

Legionnaires' disease — case follow-up

Date of interview / / Name of interviewer

Post held

Tel. no:

Personal details of case

Family name		
First name		
Age	DOB / /	Sex: ☐ Male ☐ Female
Home address		Postcode
Home telephone: ()		
GP name:		
GP address:		Postcode:
GP telephone: ()		
Patient's occupation:		
Workplace address:		Postcode:
Workplace telephone: ()		
Smoker: ☐ Yes ☐ No	Average number per day:	
Other risk factors:		
Family contacts at risk from disease: ☐ Yes ☐ No		
Name:		

Clinical details

Date of onset / /

Date of admission to hospital / /

Hospital name: _____

Ward: _____

Consultant's name: _____

Main clinical features of current illness: _____

Immunosuppression: ☐ Yes ☐ No

Cause: _____

Current illness outcome: _____

Laboratory diagnosis for this episode of illness

Urinary antigen: ☐ Yes ☐ No

Date of specimen / /

Result: ☐ Positive ☐ Negative

Culture: ☐ Yes ☐ No

Date of specimen / /

Result: ☐ Positive ☐ Negative

Serology: ☐ Yes ☐ No

Date of specimen / /

Result: ☐ Positive ☐ Negative

Organism: _____

Serogroup: _____

Risk factors for patient within incubation period (approximately 2 weeks prior to onset of illness)

Dates: / / **to** / /

Hospital inpatient: ☐ Yes ☐ No

Name of hospital: _____

Hospital outpatient: ☐ Yes ☐ No

Name of hospital: _____

Dental treatment: ☐ Yes ☐ No

Name of dentist: _____

Address: _____

Travel

Work/travel in the UK: ☐ Yes ☐ No

Usual means of transport:.

Usual route to work:

Work/travel elsewhere in UK: ☐ Yes ☐ No

Place:

Leisure

Travel abroad: ☐ Yes ☐ No

Dates of travel: / / to / /

Country:

Resort:

Hotel name:

Room no.:

Tour operator:

(Repeat for itinerary involving several different country/hotel accommodations)

Travel overnight away from home in UK: ☐ Yes ☐ No

Dates of travel: / / to / /

Town:

Hotel:

(Repeat for itinerary involving several different hotel accommodations)

Exposure risks

Showers: ☐ Yes ☐ No Place:

Air-conditioning: ☐ Yes ☐ No Place:

Fountains: ☐ Yes ☐ No Place:

Whirlpool spa/baths: ☐ Yes ☐ No Place:

Swimming pool: ☐ Yes ☐ No Place:

Other sites

Usual place(s) for shopping		Place:
Leisure centre:	☐ Yes ☐ No	Place:
Hotels:	☐ Yes ☐ No	Place:
Place of worship:	☐ Yes ☐ No	Place:
Pubs/clubs;:	☐ Yes ☐ No	Place:
Theatre/cinema/library:	☐ Yes ☐ No	Place:
Petrol stations:	☐ Yes ☐ No	Place:
Car wash:	☐ Yes ☐ No	Place:

Travel diary of patient: activities 2 weeks prior to onset

Start from day before illness and work backwards from day 1 to day 14 before illness.
List places visited and travel by bus, car, train, cycle, foot.

Day 1:	/	/
Day 2:	/	/
Day 3:	/	/
Day 4:	/	/
Day 5:	/	/
Day 6:	/	/
Day 7:	/	/
Day 8:	/	/
Day 9:	/	/
Day 10:	/	/
Day 11:	/	/
Day 12:	/	/
Day13:	/	/
Day 14:	/	/

Checklist of local places

Health/sports/leisure centres:

Swimming pools:

Hotels:

Main shopping areas/arcades:

Other specific suspect sites:

Date of completion of interview: / /

Information forwarded to:

CDSC:	☐ Yes ☐ No	Date: / /
Environmental Health Department:	☐ Yes ☐ No	Date: / /
Dept of Public Health Primary Care Trust:	☐ Yes ☐ No	Date: / /

Appendix 3 Example of a national surveillance form

This appendix provides an example from the United Kingdom (UK) of a national surveillance form.

Strictly Confidential

NATIONAL SURVEILLANCE SCHEME FOR LEGIONNAIRES' DISEASE



November 2006

CENTRES RESPONSIBLE FOR LEGIONELLA SURVEILLANCE:

Health Protection Agency Centre for Infections, Respiratory Diseases Department and Respiratory and Systemic Infection Laboratory.

OBJECTIVES:

- To detect clusters of outbreaks of legionella infection in the UK or abroad through the national surveillance of all reported cases in residents of England and Wales
- To identify sources of infection so that control measures can be applied to prevent further cases
- To disseminate legionella surveillance information to all those who need to know.

C/I USE ONLY:

CASE No. _____

CATEGORY: _____

EWGLI No. _____

REGION: _____

PLEASE RETURN THIS REPORT BY FAX OR POST TO: (insert appropriate contact details)

Personal Details

Name of patient:	Sex: Male ☐ Female ☐
Date of birth: ____/____/____	Age: _____
NHS Hospital No:	
Home address:	
Postcode:	
Occupation:	
Work address:	
	Postcode: _____

Clinical History of Case

Date of onset of symptoms of legionellosis	____/____/____
Did this patient have pneumonia?	Yes ☐ No ☐
What were the main clinical features?	
Has the patient had a recent organ transplant?	Yes ☐ No ☐ Not sure ☐
Was the patient immunosuppressed for other reasons? If Yes please give details:	Yes ☐ No ☐ Not sure ☐
Please give details of any other underlying condition:	
Hospital for patient admission:	
Date of admission:	____/____/____
Admitted to ITU:	Yes ☐ No ☐
Outcome: 1: Death ☐ (date of death: ____/____/____) 2: Still ill ☐ 3: Recovered ☐	

Hospital Acquired Case

Was the patient in hospital for any time in the two weeks BEFORE the date of onset of symptoms of legionellosis: Yes ☐ No ☐	
Hospital for patient admission:	
Diagnosis on admission:	Date of admission: ____/____/____
Type of ward or unit in which patient was resident:	
If patient was transferred from another hospital, please give details:	
Name of hospital before transfer:	
Dates of stay (from - to):	____/____/____ to ____/____/____

Possible Community Acquired Case

In the two weeks before onset of symptoms, did the patient use or spend time near a whirlpool/spa	
Yes ☐ No ☐	
If Yes, please specify:	

Possible Travel Associated Case

Did the patient spent any nights away from home (UK or abroad) in the two weeks before onset, please give details: Yes ☐ No ☐					
Country	Town or Resort	Hotel/other accommodation (apartments/campsites/cruise ships etc)	Rm No.	Dates of stay	
				Arrival	Departure
Tour Operator (if known):					
Did the patient bathe in a whirlpool/spa?			Yes ☐ No ☐ Not sure ☐		
If Yes, please specify:					
Additional information:					

Additional Information

Please provide any additional information relevant to the case's possible source of exposure. e.g. day trips, work environment:

Case Definitions for Legionnaires' disease**I) Confirmed case**

A clinical diagnosis of pneumonia with laboratory evidence of one or more of the following:

Culture of *Legionella* spp from clinical specimens;
Seroconversion (a four fold rise or greater) by the indirect immunofluorescent antibody test (IFAT) using *L. pneumophila* serogroup 1 antigen;
Positive urine ELISA using validated reagents.

II) Presumptive case

A clinical diagnosis of pneumonia with laboratory evidence of one or more of the following:

A single high titre using IFAT above;
Positive direct fluorescence (DFA) on a clinical specimen using validated monoclonal antibodies;
Seroconversion (a four fold rise or greater) by the indirect immunofluorescent antibody test (IFAT) to *L. pneumophila* other serogroups or other legionella species.

Legionella Microbiology Results

PLEASE ENSURE ALL POSITIVE SAMPLES ARE SENT TO RSIL

A: Culture: Done ☐ Not done ☐

	Date	Specimen	Species	Serogroup	Result *	
					Positive	Negative
1					☐	☐
2					☐	☐

* If positive, was the isolate referred to RSIL? Yes ☐ No ☐**B: Urine Antigen detection:** Done ☐ Not done ☐

Date	Manufacturer & Kit used	Result *	
		Positive	Negative
		☐	☐

* If positive, was the urine referred to RSIL? Yes ☐ No ☐**C: Serology:** Done ☐ Not done ☐

Date	Titre	Assay used (Manufacturer & Kit used)	Result *	
			Positive	Negative
			☐	☐
			☐	☐

* If positive, was the sera referred to RSIL Yes ☐ No ☐Has this result been confirmed in the presence of campylobacter blocking fluid? Yes ☐ No ☐ Not Sure ☐**D: Other Method: (Specify)**

Date	Specimen	Result		
		Positive	Negative	Equivocal
		☐	☐	☐

Laboratory Details

Laboratory where microbiology carried out:	
Laboratory confirmation at HPA, CFI, RSIL, Colindale?	Yes ☐ No ☐
Confirmation at another laboratory?	Yes ☐ No ☐
If Yes, please specify:	

Environmental Investigations

Has sampling of water systems been requested (see: www.hpa.org.uk/infections/topics_az/legionella/advice)	Yes ☐ No ☐
If Yes, please specify i.e. patients home, hospital, industrial/commercial, other:	Please give details:
Name and address of laboratory carrying out sampling:	
Results of sampling (if known):	Pos ☐ Neg ☐ Not Known ☐

Reporter's Details

Name of person reporting case to CDSC:	
Date of report:	__/__/__
Telephone contact number:	
Email address:	
Name of CCDC relevant to case:	
Name of HPU responsible for reporting case:	

Signature: Date:

Glossary

aerobic bacteria	Bacteria that require the presence of free or dissolved oxygen in their environment for survival and reproduction.
aerosol	A suspension of fine solid or liquid particles in a gas, such as air.
anaerobic bacteria	Bacteria that live and reproduce in an environment that contains no free or dissolved oxygen.
antibody	A protein produced by the body's immune system that recognizes and helps fight infections and other foreign substances in the body.
antigen	A foreign substance that stimulates the production of antibodies by the immune system.
ascertainment	The determination through diagnostic methods of whether or not a person is infected with the disease.
aspiration	The inhalation of foreign material, such as food or airborne particles, into the lung.
biofilm	A slimy matrix produced and inhabited by bacteria, which enables the bacteria to adhere to a surface and carry out certain essential biochemical processes.
blow-down (or bleed-off)	Removing some of the water of a system periodically or continuously, and replacing it with fresh water, to control the continuous accumulation of dissolved solids in the water.
bronchoalveolar lavage	Washing the bronchial tubes and alveoli with repeated injections of water.
community acquired	Cases of legionellosis that are not acquired in a health-care, travel or domestic (i.e. the patient's home) setting.
comorbidity	A disease or disorder that is not directly caused by another disorder but occurs at the same time.
control measure	Any action and activity that can be used to prevent or eliminate a water safety hazard or reduce it to an acceptable level.
control point	A step at which control can be applied to prevent or eliminate a water safety hazard or reduce it to an acceptable level. Some plans contain key control points; that is, points at which control might be essential to prevent or eliminate a water safety hazard.
corrective action	Any action to be taken when the results of monitoring at the control point indicate a loss of control.
deadleg	A length of water-filled pipe where there is little or no flow.
decimal reduction time	A unit of microbial heat resistance, defined as the time required to kill 90% of a population of microorganisms at a constant temperature, under specified conditions.

definite nosocomial	Legionnaires' disease in a person who was in hospital for 10 days before the onset of symptoms.
domestically acquired	Cases of legionellosis acquired in patients' homes.
drift	Water droplets that are generated within a device (such as a cooling tower or evaporative condenser), and carried in the airflow without initial evaporation.
drift eliminator	An inertial stripping device used to remove water droplets.
ecology	The relationship between an organism and its environment.
endotoxin	A substance found in the cell walls of Gram-negative bacteria that can be extremely toxic to people, producing fever, shock, and even death.
epitope	Part of a foreign organism or its proteins that is recognized by the immune system and targeted by antibodies, cytotoxic T cells or both.
evaporative condenser	Heat-transfer device, in which warm water is cooled by evaporation in atmospheric air (also known as an evaporative fluid cooler or closed circuit cooling tower).
extrapulmonary syndrome	Caused when <i>Legionella pneumophila</i> spreads from the respiratory system to the body (usually the heart, but also the spleen, liver, kidney, bone and bone marrow, joints, inguinal and intrathoracic lymph nodes and digestive tract). Extremely rare.
flow diagram	A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular item.
Gram stain	A technique used to identify bacteria, in which a violet dye — followed by a red dye — is used to stain bacterial cell walls. Gram-positive bacteria retain the violet dye; Gram-negative bacteria appear red.
greywater	Domestic wastewater that does not contain human wastes, such as bath, shower, or washing machine water (also referred to as <i>sullage</i>).
haemoptysis	Coughing up blood.
hazard	In the context of this document, a biological, chemical or physical agent in water, or a condition of water, with the potential to cause an adverse health effect.
hazard analysis	The process of collecting and evaluating information on hazards and conditions leading to their presence, for the purpose of deciding which are significant for water safety and therefore should be addressed in a water safety plan.
health-based target	Target based on critical evaluation of health concerns; for example, a target might be "no cases of legionellosis caused by artificial water systems".

health-care acquired	Legionellosis that is acquired in a health-care setting (sometimes referred to as nosocomial).
heterotroph	An organism that is incapable of making its own food, but must feed upon organic compounds produced by other organisms.
heterotrophic plate count (HPC)	A test used to estimate the total number of all types of bacteria in an environmental sample, usually water. The lower the HPC, the better the biological water quality. Other names for the procedure (within the water industry) include total plate count, standard plate count, plate count and aerobic plate count.
hot tub	A facility that is designed for sitting in (rather than swimming), contains treated water usually above 32 °C, is usually aerated, and is not drained, cleaned or refilled for each user. Also known as a spa pool, whirlpool, whirlpool spa, heated spa, bubble bath or Jacuzzi. (See also <i>whirlpool bath</i> and <i>natural spa</i> .)
incubation period	The time interval between initial exposure to infection and appearance of the first symptom or sign of disease.
intubation	Insertion of a tube into the trachea to assist with breathing.
<i>Legionella</i> -like amoebal pathogen (LLAP)	<i>Legionella</i> that cannot be grown on routine <i>Legionella</i> culture media, but that replicate within the cytoplasm of amoebae.
Legionnaires' disease	The most severe and common form of pneumonia caused by <i>Legionella pneumophila</i> . Symptoms are nonspecific; however, the disease has a rapid onset and can be fatal.
legionellosis	Generic term used to describe infections caused by <i>Legionella pneumophila</i> , which can range in severity from a mild, febrile illness (Pontiac fever) to a rapid and potentially fatal pneumonia (Legionnaires' disease).
monitor	The act of conducting a planned sequence of observations or measurements of control parameters, to assess whether a control point is under control.
natural spa	Facility containing thermal or mineral water, which may be perceived to have therapeutic value; because of certain water characteristics, a natural spa may receive minimal water quality treatment. See also <i>hot tub</i> .
nosocomial	Legionellosis that is acquired in a health-care setting (usually referred to as health-care acquired). See also <i>definite nosocomial</i> , <i>probable nosocomial</i> and <i>possible nosocomial</i> .
opportunistic bacteria	Bacteria that take advantage of certain conditions (e.g. a host's lowered immunity) to cause disease.
outbreak	Two or more confirmed cases of legionellosis occurring in the same hospital or residential institution within a six-month period.
pathogenicity	Capacity to cause disease.

performance targets	Goals for water quality; typically expressed in terms of required reductions of a substance of concern, or effectiveness in preventing contamination.
pleura	A thin membrane that covers the lungs (visceral pleura) and lines the chest cavity (parietal pleura).
pleural effusion	A collection of fluid inside the chest cavity around the lung.
pleural space	Also known as the pleural cavity, this is the area between the pleura (see above). The pleural space is normally filled with fluid.
polymicrobial	Characterized by the presence of several species of microorganisms.
Pontiac fever	The mildest form of legionellosis (caused by <i>Legionella pneumophila</i>); usually self-limited and typically does not require treatment.
possible nosocomial	Legionnaires' disease in a person who was in hospital 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 (or the residential institution).
probable nosocomial	Legionnaires' disease in a person who was in hospital 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 (by monoclonal antibody subgrouping or by molecular typing methods) from isolates obtained from the hospital water system at about the same time.
prognosis	A prediction of the probable course and outcome of a disease.
sentinel point	Point in a water system that poses the highest risk from infection (e.g. the furthest point from the water heater in a hot water system, or the incoming water in a cold water system).
sequela	A pathological condition resulting from a disease.
seroconversion	Development of antibodies in blood serum as a result of infection or immunization.
serogroup	A subdivision of a species or subspecies distinguishable from other strains therein on the basis of antigenic character testing for recognizable antigens on the surface of the microorganism.
shot dose	A brief, high-level treatment.
sporadic	An isolated or unique case of a disease.
sullage	Domestic wastewater other than that from toilets (also referred to as <i>greywater</i>).

surveillance	The process of systematic collection, orderly consolidation, and analysis of data, with prompt dissemination and feedback of the results to those who need to know, particularly those who are in a position to take action.
travel-associated	Cases of legionellosis acquired during travel (e.g. from a cruise ship or a hotel).
treatment target	Direct specification of acceptable technologies for specific circumstances.
validation	The process of obtaining accurate and reliable evidence that a water safety plan is effective.
verification	The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine compliance with a water safety plan.
virulence	Degree of an organism's ability to cause disease, as indicated by mortality rate from the related disease, or its ability to invade tissues and cause disease.
water safety plan	A comprehensive risk assessment and risk management approach that encompasses all steps in water supply, from catchment to consumer.
whirlpool bath	Type of hot tub sometimes found in bathrooms of hotel rooms or private residences. The bath is fitted with high-velocity water jets or air injection, and the water is emptied after each use. See also <i>hot tub</i> .

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