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# StandardGuide for the Inspection of Water Systems for Legionella and the Investigation of Possible Outbreaks of Legionellosis (Legionnaires' Disease or Pontiac Fever)[1]

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## 1. Scope

1.1 This guide covers appropriate responses for employers, building owners and operators, facility managers, health and safety professionals, public health authorities, and others: (*1*) to a concern that a water system may be contaminated with the bacterium known as legionella (see 6.1); and (*2*) to the identification of one or more cases of Legionnaires' disease or Pontiac fever (see 6.3-6.5). Comprehensive and explicit recommendations to limit legionella multiplication in water systems, disinfect potential sources of human exposure to legionella, and prevent health-care associated infections are beyond this guide's scope.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* See 7.3 and 8.5 for specific hazard statements.

## 2. Referenced Documents

2.1 *ASTM Standards:*[2]

C1080 Specification for Asbestos-Cement Products Other Than Fill For Cooling Towers
D512 Test Methods for Chloride Ion In Water
D596 Guide for Reporting Results of Analysis of Water
D887 Practices for Sampling Water-Formed Deposits
D1067 Test Methods for Acidity or Alkalinity of Water
D1129 Terminology Relating to Water
D1293 Test Methods for pH of Water
D1356 Terminology Relating to Sampling and Analysis of Atmospheres
D2331 Practices for Preparation and Preliminary Testing of Water-Formed Deposits
D3370 Practices for Sampling Water from Closed Conduits
D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water
D4840 Guide for Sample Chain-of-Custody Procedures
E645 Test Method for Efficacy of Microbicides Used in Cooling Water Systems
F444 Consumer Safety Specification for Scald-Preventing Devices and Systems in Bathing Areas
F445 Consumer Safety Specification for Thermal-Shock-Preventing Devices and Systems in Showering Areas

2.2 *APHA Documents:*[3]

Public Health Law Manual, Third Edition
Standard Methods for the Examination of Water and Wastewater, Twenty-first Edition
Control of Communicable Diseases Manual, Eighteenth Edition

2.3 *ASHRAE Documents:*[4]

Codes and Standards. 2004 ASHRAE Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment
Cooling Towers. 2004 ASHRAE Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment
Water Treatment. 2004 ASHRAE Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment
12–2000 Minimizing the Risk of Legionellosis Associated with Building Water Systems
62.1-2007 ASHRAE Standard. Ventilation for Acceptable Indoor Air Quality

[1] This guide is under the jurisdiction of ASTM Committee D22 on Air Qualityand is the direct responsibility of Subcommittee D22.05 on Indoor Air.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from the American Public Health Association, 800 I St. N.W., Washington, DC 20001, USA, http://www.apha.org.

[4] Available from the American Society of Heating, Refrigerating, and Air-Conditioning Engineers, Inc. (ASHRAE), 1791 Tullie Circle, NE, Atlanta, GA 30329, USA, http://www.ashrae.org.

2.4 *ASM Documents:*
Manual of Clinical Microbiology, Ninth Edition[5]
Manual of Environmental Microbiology, Third Edition[6]
Manual of Molecular and Clinical Laboratory Immunology, Seventh Edition[7]

2.5 *AWT Document:*[8]
*Legionella* 2003: An Update and Statement by the Association of Water Technologies (AWT)

2.6 *CDC Documents:*[9]
2000 Guidelines for Preventing Opportunistic Infections Among Hematopoietic Stem Cell Transplant Recipients
2003 Guidelines for Environmental Infection Control in Health-Care Facilities
2003 Guidelines for Preventing Health-Care-Associated Pneumonia
2005 Procedures for the Recovery of Legionella from the Environment
2005 Case Definition for Legionellosis (*Legionella pneumophila*)

2.7 *Code of Federal Regulations:*[10]
Title 42, Volume 1, 84. Approval of Respiratory Protective Devices

2.8 *CTI Document:*[11]
Legionellosis Guideline: Best Practices for Control of Legionella

2.9 *OSHA Document:*[12]
2003 Occupational Safety and Health Administration (OSHA) Technical Manual, Section III: Chapter 7, Legionnaires' Disease

2.10 *WHO Document:*[13]
Legionella and the Prevention of Legionellosis

## 3. Terminology

3.1 *Definitions from Compilation of ASTM Standard Definitions.*

3.1.1 *aerosol, n*—a dispersion of solid or liquid particles in a gaseous medium.

3.1.2 *air conditioning, n*—the simultaneous control of all, or at least the first three, of those factors affecting both the physical and chemical conditions of the atmosphere within any structure. These factors include temperature, humidity, motion, distribution, dust, bacteria, odor, and toxic gases.

3.1.3 *biocide, n*—any chemical intended for use to kill organisms.

3.1.4 *biofilm, n*—an accumulation of cells immobilized on a substratum and frequently embedded in an organic polymer matrix of microbial origin.

3.1.5 *cooling tower, n*—a structure used to dissipate heat in open recirculating cooling systems.

3.1.6 *exposure, n*—contact with a chemical, biological, physical, or other agent over a specified time period.

3.1.7 *inspection, n*—the process of measuring, examining, testing, gaging, or otherwise evaluating materials, products, services, systems, or environments.

3.1.8 *monitoring, n*—the continual sampling, measuring, recording, or signaling, or both, of the characteristics of water or waterborne material.

3.1.9 *pH, n*—the negative logarithm of hydrogen-ion activity in aqueous solution or the logarithm of the reciprocal of the hydrogen-ion activity.

3.1.10 *sample, n*—a portion of a population intended to be representative of the whole.

3.1.11 *sampling, n*—a process consisting of the withdrawal or isolation of a fractional part of the whole.

3.1.12 *scale, n*—a deposit formed from solution directly upon a surface.

3.1.13 *sludge, n*—a water-formed sedimentary deposit.

3.1.14 *testing, n*—the determination by technical means of properties; performance; or elements of materials, products, services, systems, or environments which involve application of established scientific principles and procedures.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *acute phase, n— of legionellosis*, the initial phase of infection; the first weeks following symptom onset.

3.2.2 *antibody, n—to legionella*, a substance in blood synthesized in response to a legionella antigen that enters the body.

3.2.3 *antibody rise, n—in legionella antibody*, an increase in the highest serum dilution at which legionella antibody is detected in a blood sample collected weeks or months after legionellosis onset as compared with the highest dilution for a sample collected before or shortly after illness onset.

3.2.4 *antigen, n—to legionella*, a legionella molecule that stimulates an antibody response by a host immune system.

3.2.5 *aseptically, adv*—using precautions to prevent contamination of samples by microorganisms.

3.2.6 *back-flow preventer, n*—a control valve to prevent reverse flow of water.

3.2.7 *bacterium, n—pl. -ria*, a typically small unicellular microorganism.

[5] Edelstein, P.H., "*Legionella*," in *Manual of Clinical Microbiology* , Murray, P.R., Ed., American Society for Microbiology, Washington, DC 20005, USA, 2007, pp. 835–849.

[6] Fields, B.S., "Legionellae and Legionnaires' disease" in *Manual of Environmental Microbiology*. Hurst, C.J., Ed., American Society for Microbiology, Washington, DC 20005, USA, 2007, pp. 1005–1015.

[7] Edelstein, P.H., "Detection of Antibodies to Legionella," in *Manual of Molecular and Clinical Laboratory Immunology*. Detrick, B., Hamilton, R.G., Folds, J.D., Eds., American Society for Microbiology, Washington, DC 20005, USA, 2006, pp. 468–476.

[8] Available from the Association of Water Technologies, 15245 Shady Grove Rd., Rockville, MD 20850, USA, http://www.awt.org, 2003.

[9] Available from the U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333, USA, http://www.cdc.gov.

[10] Available from the U.S. Government Printing Office, 732 N. Capitol St., N.W., Washington DC, 20401, USA, 42CFR84, 2004.

[11] Available from the Cooling Tower Institute, P.O. Box 73383, Houston, TX, USA, http://www.cti.org, 2006.

[12] Available from Occupational Safety and Health Administration (OSHA), 200 Constitution Ave., NW, Washington, DC 20210, USA, http://www.osha.gov.

[13] Available from the World Health Organization, Geneva, Switzerland, http://www.who.int/en/, 2007.

3.2.8 *CDC, n*—Centers for Disease Control and Prevention, U.S. Public Health Service, Atlanta, Georgia.

3.2.9 *clean, adj*—visibly free of sludge, sediment, scale, biofilm, algae, fungi, rust, corrosion, and extraneous matter.

3.2.10 *clean, v*—to remove sludge, sediment, scale, biofilm, algae, fungi, rust, corrosion, and extraneous matter by physical or chemical means.

3.2.11 *colony, n—of legionella*, a macroscopic group of legionella cells arising from bacterial multiplication on the surface of semisolid culture medium.

3.2.12 *colony-forming unit, n—of legionella*, a colony arising from the multiplication of one or a cluster of viable legionella.

3.2.13 *confirmed case, n—of Legionnaires' disease*, a case of physician-diagnosed pneumonia verified by at least one confirmatory test as meeting the laboratory criteria jointly developed by the CDC and the Council of State and Territorial Epidemiologists.

3.2.14 *contamination, n—with legionella*, the presence of legionella on or in inanimate articles or substances.

3.2.15 *convalescent phase, n—of legionellosis*, the recovery phase of infection, typically four to eight weeks following symptom onset.

3.2.16 *DFA, adj*—direct fluorescent-antibody.

3.2.17 *dead leg, n*—a length of pipe closed at one end or ending at a fitting through which water flows only when the fitting is open.

3.2.18 *direct fluorescent-antibody test, n—for legionella*, a staining procedure that detects legionella surface antigens through the use of specific antibodies labeled with fluorescent compounds; bacteria to which antibody has attached fluoresce when viewed under appropriate irradiation.

3.2.19 *disinfect, v*—to eliminate virtually all pathogenic microorganisms, but not necessarily all microbiological forms, outside the body by direct exposure to chemical or physical agents.

3.2.20 *drift, n—from water-cooled heat-transfer equipment*, water droplets carried from a cooling tower or other water-cooled heat-transfer system by air movement through the unit; drift can be confused with condensed water vapor appearing as steam leaving a unit.

3.2.21 *drift eliminator, n*—a plastic, metal, or wood baffle designed to entrain water droplets and to reduce aerosol escape.

3.2.22 *evaporative condenser, n*—a heat exchanger in which refrigerant is cooled by a combination of air movement and water spraying.

3.2.22.1 *Discussion*—Evaporative air coolers (swamp coolers), which do not produce large numbers of water droplets, have not been associated with legionella transmission to date.

3.2.23 *exhaust outlet, n—in a ventilation system*, an outlet from which an air-handling system discharges air outdoors.

3.2.24 *false-negative, adj*—incorrectly indicating the absence of a finding, condition, or disease.

3.2.25 *false-positive, adj*—incorrectly indicating the presence of a finding, condition, or disease.

3.2.26 *free residual chlorine, n*—the total concentration of hypochlorous acid and hypochlorites available to act as disinfectant.

3.2.27 *genus, n*—a taxonomic classification of organisms; the division between the family or tribe and the species; a group of species alike in broad organizational features but different in detail.

3.2.28 *gram-negative, adj*—losing the primary violet or blue stain during decolorization in Gram's staining method.

3.2.29 *HVAC, adj*—heating, ventilating, and air-conditioning.

3.2.30 *humidifier, n*—a device for adding moisture to air by boiling, spraying, or atomizing water.

3.2.31 *IHC, n*—immunohistochemistry.

3.2.32 *immunocompromised, adj*—a person's state when the body's natural defenses to infection are below normal.

3.2.33 *immunohistochemistry, n*—a staining procedure that detects antigens in tissue sections through the use of specific labeled antibodies.

3.2.34 *in vitro, adj*—(Latin: in glass), refers to laboratory tests performed in a test tube or other container as opposed to a living system; the opposite of *in vivo*.

3.2.35 *in vivo, adj*—(Latin: in living), refers to laboratory tests performed in living organisms; the opposite of *in vitro*.

3.2.36 *incubation period, n— of legionellosis*, the time interval between initial contact with legionella and appearance of the first legionellosis sign or symptom.

3.2.37 *infection, n—with legionella*, the entry and development, or multiplication, of legionella in humans.

3.2.38 *inspector, n*—a person examining an environment for possible contamination with legionella.

3.2.39 *investigator, n*—a person conducting an epidemiological investigation of a potential legionellosis outbreak.

3.2.40 *isolate, n*—a microorganism grown from a clinical or environmental sample.

3.2.41 *isolate, v—in vitro* growth of microorganisms on culture medium.

3.2.42 *Legionella, n*—a bacterial genus containing over 50 species and at least 71 serogroups; abbreviated to the first initial when used repeatedly with a species name, for example, *L. pneumophila*.

3.2.43 *legionella, n—pl. -ae*, a bacterium in the genus *Legionella*.

3.2.44 *legionellosis, n*—a respiratory illness caused by or associated with legionella; two forms of legionellosis due to inhalation of airborne legionella are recognized, that is, Legionnaires' disease and Pontiac fever.

3.2.45 *Legionnaires' disease, n*—an illness characterized by pneumonia and caused by or associated with legionella infection, most often *L. pneumophila*.

3.2.46 *maintain, v*—to perform regular and routine activities aimed at preserving equipment, operational standards, and cleanliness; includes inspection, repair, preventive servicing, and cleaning.

3.2.47 *maintenance program, n*—the assembly of relevant data and the setting out of a formal strategy and recording system for effective management of a series of maintenance procedures.

3.2.48 *make-up water, n*—fresh water added to a circulating water system to compensate for losses due to evaporation, purging, drift, or leakage.

3.2.49 *microorganism, n*—a microscopic organism.

3.2.50 *N95 filtering facepiece respirator, n*—a device that has met the requirements of 42 Code of Federal Regulations, Part 84, to protect the wearer against inhalation of a harmful atmosphere and provides a minimum of 95 % filter efficiency against certain solid and non-oil-based particles.

3.2.51 *opportunistic infection, n*—an infection caused by normally nonpathogenic organisms in a host whose resistance has been decreased.

3.2.52 *outbreak, n—of legionellosis*, the occurrence of two or more confirmed legionellosis cases in a limited time period (for example, weeks to months) and geographic region (for example, a building, limited area within a building, or up to several kilometres around a potential source); the occurrence of cases in excess of the number expected in a given time period and locale.

3.2.53 *outdoor air intake, n— for ventilation systems*, an opening through which outdoor air is introduced into a building's air-handling system.

3.2.54 *PCR, adj*—polymerase chain reaction.

3.2.55 *polymerase chain reaction test, n*—a technique for the selection and amplification of specific genetic sequences.

3.2.56 *Pontiac fever, n*—a self-limited, short-duration, non-fatal disease characterized by fever and cough caused by or associated with legionella.

3.2.57 *protozoan, n—pl. -a*, single-celled microorganism representing the lowest form of animal life.

3.2.58 *sensitivity, n— of a test for legionellosis or legionella*, a method's ability to accurately detect the presence of the disease being tested (that is, legionellosis) or a causative agent (that is, a legionella).

3.2.59 *serogroup, n—of legionella*, a subgroup within a legionella species.

3.2.60 *serology, n*—the study of blood serum for evidence of infection, performed by evaluation of antigen-antibody reactions *in vitro*.

3.2.61 *serum, n—pl. -a*, the clear, thin, sticky fluid portion of blood remaining after coagulation.

3.2.62 *source, n—of legionella*, the water system, supply, or equipment from which legionella pass to a host.

3.2.63 *species, n*—a taxonomic classification of organisms; the division between genus and variety or individual; a group of organisms bearing a close resemblance in essential organizational features.

3.2.64 *specificity, n— of a test for legionellosis or legionella*, a method's ability to identify accurately an illness as legionellosis or a bacterium as a legionella; a method's ability to select and distinguish legionella from all other bacteria in the same environment.

3.2.65 *sporadic case, n—of legionellosis*, an occurrence of legionellosis apparently independent of other cases.

3.2.66 *subtype, n—of legionella*, a subgroup within a legionella serogroup.

3.2.67 *surveillance, n— of legionellosis*, the continuing scrutiny of aspects of the occurrence and spread of legionellosis that are pertinent to effective control.

3.2.68 *susceptibility, n—to legionellosis*, the state of not possessing sufficient resistance against legionella to prevent infection or disease, if or when, exposed to the bacterium.

3.2.69 *titer, n—in legionellosis serology*, the highest serum dilution at which a test detects legionella antibody.

3.2.70 *viable, adj*—capable of living or replicating under a given set of growth conditions; usually determined by isolation of legionella on culture medium, that is, *in vitro*, or in laboratory animals, that is, *in vivo*.

3.3 Refer to Terminology D1129 and Terminology D1356 for definitions of other terms used in this guide.

## 4. Summary of Guide

4.1 Section 6 of this guide provides background information on (*1*) legionella bacteria; (*2*) microbiological analysis of environmental samples for legionella; and (*3*) recognition and diagnosis of legionellosis. Section 7 describes environmental inspections of water systems for legionella and suggests general control measures to limit legionella multiplication. Section 8 explains how to collect environmental samples to detect the presence of legionella. Section 9 outlines an epidemiological investigation of a possible legionellosis outbreak. Section 10 recommends control measures for (*1*) water-cooled heat-transfer systems; (*2*) potable hot and cold water supplies; (*3*) heating, ventilating, and air-conditioning (HVAC) systems; (*4*) spas, whirlpool baths, and jacuzzis; and (*5*) decorative fountains. This guide uses the term *inspector* when referring to a person examining the environment for possible legionella contamination (see Section 7) and the term *investigator* when referring to a person conducting an epidemiological study of a possible legionellosis outbreak (see Section 9). Inspection and investigation teams may include public health authorities, corporate or institutional health-care providers, building owners and operators, facility managers, employee representatives, and public or private health and safety professionals.

## 5. Significance and Use

5.1 Water systems may be inspected (see Section 7) and tested (see Section 8) for legionella under three circumstances (*1*) in the absence of reported legionellosis (see 5.2); (*2* ) when

a single legionellosis case has been reported (see 5.3); and (*3*) when two or more legionellosis cases are reported in a limited time period and geographic region (see 5.4). Following are factors building owners and operators need to understand when considering testing water systems for legionella in the absence of illness (see 5.2) and for single legionellosis cases (see 5.3). Refer also to the CDC 2003 Guidelines for Preventing Health-Care Associated Pneumonia, and the CDC 2000 Guidelines for Preventing Opportunistic Infections Among Hematopoietic Stem Cell Transplant Recipients, and the WHO Legionella and the Prevention of Legionellosis. Detection of legionella in a water system is not sufficient to identify the system as a health hazard. However, failure to detect legionella does not indicate, conclusively, that the bacterium is not present (see 6.2.4) or that the water system may not pose a potential health hazard. Methods to detect legionella vary in sensitivity and specificity (see 6.2), and laboratories vary in their skill and experience in the isolation and identification of legionella. Isolation of apparently identical legionellae from clinical and environmental samples (see 6.2.1, 6.6.2.4, and Section 8) may suggest that a water system was the source of the legionella responsible for a patient's infection (see 5.3.2). However, cases of Legionnaires' disease due to different legionella serogroups or species need not necessarily have different sources of exposure because a system may be contaminated by more than one legionella. Timely inspection, testing, and treatment of possible legionella sources may reduce legal liabilities for facility owners and operators. Refer also to the APHA Public Health Law Manual.

5.2 *Environmental Testing for Legionella in the Absence of Illness:*

5.2.1 Concerned employers, building owners and operators, facility managers, and others seek to prevent real and potential health hazards, if possible. Water system operators may identify undesirable situations by monitoring routinely for legionella and may be able to implement control measures before the bacterium reaches an amount sufficient to cause human illness (see 6.2.4.2). The CDC 2000 Guidelines for Preventing Opportunistic Infections Among Hematopoietic Stem Cell Transplant Recipients advises that because transplant recipients are at much higher risk for disease and death from legionellosis compared with other hospitalized persons, periodic culturing for legionella in water samples from a center's potable water supply could be regarded as part of an overall strategy for the prevention of Legionnaires' disease in transplant centers and other facilities housing persons at high risk of infection if exposed (see 6.4.2). There is some evidence that environmental legionella surveillance should be considered a proactive strategy for the prevention of hospital-acquired Legionnaires' disease (**1**). However, the optimal methodology (that is, frequency or number of sites) for environmental surveillance cultures in transplant centers has not been determined, and the cost-effectiveness of such a strategy has not been evaluated for either transplant centers or other health-care settings nor for institutional, commercial, or residential buildings.

5.2.2 Some experts advise against testing water systems for legionella in the absence of illness, particularly in buildings other than hospitals or health-care facilities, given that absolute exclusion of this bacterium from water systems may not be necessary to prevent legionellosis nor may it be achievable without considerable expense. Microbiological water monitoring increases operational costs, and interpretation of test results may be difficult (see 6.2.4). Identification of legionella in environmental samples also may cause unwarranted alarm and unnecessary remediation. The WHO publication states that legionella testing cannot be considered a control measure, but does provide some evidence that the water safety plan is effective and that control measures are operating properly. Sampling for legionella cannot provide results sufficiently quickly to be useful in operational monitoring, which instead should be by measures that provide real-time results, for example, monitoring of the biocide concentration, temperature, and pH of the water.

5.3 *Environmental Testing for Legionella for a Single (Sporadic) Legionellosis Case:*

5.3.1 Testing potential legionella sources as soon as possible after confirmation of legionellosis may increase the likelihood of identifying the responsible source. Environmental conditions and equipment operation may change frequently, which may affect the likelihood of legionella detection. Inspectors may fail to identify the responsible source if they postpone sampling until an illness is confirmed as legionellosis (see 6.6 and 6.7) or until a search for other cases identifies common exposures (see Section 9).

5.3.2 Persons with legionellosis often have been exposed to more than one possible source during the disease's incubation period (see 6.4.3, 6.5.3) and may not recognize or recall all possible exposures. Isolation of apparently identical legionellae from clinical and environmental samples (see 6.2.1, 6.6.2.4, and Section 8) is suggestive, but does not identify a source absolutely as the site of a patient's exposure because the distribution of legionella species, serogroups, and subtypes (see 6.1.1 and 6.1.2) in the environment is not known, that is, the same legionella could colonize more than one water system. Identification of the environmental source responsible for legionella transmission may be difficult if no clinical isolate is available for comparison with environmental isolates (see 6.2.1, 6.6.2.4). Legionella has been found in a substantial proportion of water systems tested in prevalence surveys and outbreak investigations. Without a clinical isolate, identification of the probable source of legionella transmission must be based on environmental and epidemiological information (see Sections 7-9).

5.4 *Environmental Testing for Legionella for Multiple Legionellosis Cases*—Identification of multiple legionellosis cases in a circumscribed area and limited time period or that share a potential source warrants (*1*) environmental inspection of suspect sources to identify the water system responsible for legionella transmission to prevent further illness (see Sections 7-9); and (*2*) epidemiological investigation to identify common risk factors for cases (see 6.4.2, 6.5.2). Information from an epidemiological investigation (see Section 9) often facilitates identification of specific environments the legionellosis patients shared and on which inspectors should focus attention (see Sections 7 and 8). Environmental testing supplements, but does not replace, inspection and prompt correction of identified

problems (see Section 10) at all possible legionella sources regardless of whether or not legionella is detected or the potential source is implicated in patient exposure.

## 6. Background

6.1 *Legionella*—Refer to the APHA Standard Methods for the Examination of Water and Wastewater, the ASM Manual of Clinical Microbiology, the ASM Manual of Environmental Microbiology, the WHO Legionella and the Prevention of Legionellosis, and Refs (**2 and 3**) for background information on legionella.

6.1.1 *The Genus Legionella*—The legionella family is a diverse group of mesophilic, motile, obligately aerobic, nutritionally fastidious, poorly staining, gram-negative, rod-shaped bacteria. Microbiologists currently recognize over 50 species in this genus of which approximately one half have been associated with human illness. The genus name *Legionella* is abbreviated when used repeatedly with species names, for example, *Legionella pneumophila* is written as *L. pneumophila*. Microbiologists can distinguish serogroups, identified by number, within some legionella species, for example, *L. pneumophila* Serogroup 1. Some serogroups can be separated further into subtypes.

6.1.2 *Pathogenic Legionella—L. pneumophila* (in particular Serogroup 1) accounts for more than 90 % of legionellosis cases that have been studied in the United States. Other species associated with clinical infections include *L. micdadei, L. dumoffii, L. bozemanii*, and *L. longbeachae*. It is likely that most *Legionella* species can cause human disease under appropriate conditions; however, such infections are reported infrequently because they are rare and diagnostic reagents are lacking. Some legionellae cannot be grown on routine legionella medium and have been termed *Legionella*-like amebal pathogens, of which at least one is considered a human pathogen.

6.1.3 *Legionella in the Environment*—Legionella is found worldwide in a variety of natural and man-made aquatic environments, usually ones with moderately elevated temperatures (see 6.1.4, 6.3.4, 7.3.6). Legionella lives in biofilms near the surfaces of lakes, rivers, and streams and in conjunction with specific free-living protozoa.

6.1.4 *Legionella in Man-Made Water Systems*—Factors known to enhance legionella colonization of man-made water systems (see 6.1.3 and 6.3.4) include warm temperature (25 to 45°C), suitable pH (2.5 to 9.5), and water stagnation followed by agitation, as well as the presence of other organisms, sediment, and scale (see 6.1.3, 6.1.5). It is uncommon to find legionella proliferation at water temperatures below 20°C and the bacterium does not survive in waters warmer than 60°C. Chlorination of potable water supplies may not eradicate legionella (see 6.1.5). Low concentrations of legionella (even below concentrations detectable by conventional test methods, see 6.2) can colonize water systems and can multiply under suitable conditions. Monochloramine rather than chlorine disinfection of municipal water supplies may reduce legionella transmission (**4**, **5**).

6.1.5 *Association of Legionella with Other Organisms*—In humans, legionella infects alveolar macrophages, a type of white blood cell in the lungs. In the environment, the bacterium infects free-living aquatic amebae and other protozoa (see 6.1.3 and 6.1.4). Legionella inside protozoa may be protected from biocides, desiccation, and other environmental stresses.

6.2 *Microbiological Analysis of Environmental Samples for Legionella*—Legionella can be detected in environmental samples by three methods (*1*) growth of viable bacteria on culture medium (see 6.2.1); (*2*) detection of legionella cells with a direct fluorescent-antibody (DFA) stain (see 6.2.2); and (*3*) detection of legionella genetic material with a polymerase chain reaction (PCR) test (see 6.2.3). DFA and PCR results are available sooner than culture, but isolation is the standard or primary laboratory method to detect legionella (see 6.2.1) because it provides information on bacterial viability (necessary for infection) and allows more thorough bacterial characterization (necessary for outbreak investigation and source identification) (see 6.2.1.2). Legionella cells in water samples and washings of other materials (see Section 8) typically are concentrated by filtration or centrifugation before testing. Detection limits for these methods depend on the source material, volume of sample analyzed, and analytical method. Refer to Guides D596 and D3856, Practice D2331, the APHA Standard Methods for the Examination of Water and Wastewater, the CDC 2005 Procedures for the Recovery of Legionella from the Environment, and the WHO Legionella and the Prevention of Legionellosis for information on the detection and identification of legionella from environmental samples.

6.2.1 *Legionella Isolation:*

6.2.1.1 *Primary Isolation*—Water samples and washings of other materials (see Section 8) may be treated with heat or buffered acid solution to reduce the numbers of nonlegionella organisms prior to inoculation of culture medium; specificity: 100 %; sensitivity: varies with water source and sample handling. Preliminary culture results typically are not available for three to five days after sample receipt because the method depends on bacterial multiplication into visible colonies. Some legionellae may not form visible colonies for 10 to 14 days. Confirmation of culture results may require an additional three to five days following primary isolation. Hold primary plates for at least 14 days before reporting them as negative, that is, no legionella isolated.

6.2.1.2 *Isolate Identification*—The specific species, serogroup, and subtype to which an environmental legionella isolate belongs may be identified with a DFA test (see 6.2.2 and 6.6.2.2) or by biochemical or nucleic acid analyses. Laboratories should preserve any environmental legionella isolates from outbreak investigations to allow further examination by public health authorities and for more specific identification by methods that may not be available commercially (see 5.3.2 and 6.6.2.4).

6.2.2 *Direct Fluorescent-Antibody (DFA) Test*—Microbiologists can detect bacteria in environmental samples with DFA stains similar to those used to identify culture isolates (see 6.2.1.2 and 6.6.2.4) and to detect legionella directly in clinical specimens (see 6.6.2.2). However, DFA stains react with both living and dead legionella cells and may stain other bacteria. Contaminants in specimen containers and