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Standard Guide for Inspecting Water Systems for Legionellae and Investigating Possible Outbreaks of Legionellosis (Legionnaires' Disease or Pontiac Fever)[1]

This standard is issued under the fixed designation D 5952; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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 bacteria known as legionellae (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 and to disinfect potential sources of human exposure to legionellae 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]

D 512 Test Methods for Chloride Ion in Water
D 596 Practice for Reporting Results of Analysis of Water
D 887 Practices for Sampling Water-Formed Deposits
D 1067 Test Methods for Acidity or Alkalinity of Water
D 1129 Terminology Relating to Water
D 1192 Specification for Equipment for Sampling Water and Steam in Closed Conduits
D 1293 Test Methods for pH of Water
D 1356 Terminology Relating to Sampling and Analysis of Atmospheres
D 2331 Practices for Preparation and Preliminary Testing of Water-Formed Deposits
D 3370 Practices for Sampling Water from Closed Conduits
D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water
D 4840 Guide for Sample Chain-of-Custody Procedures
E 645 Test Method for Efficacy of Microbicides Used in Cooling Systems

2.2 *APHA Documents:*[3]

The Public Health Law Manual, Second Edition
Standard Methods for the Examination of Water and Wastewater, Twentieth Edition
Control of Communicable Diseases Manual, Seventeenth Edition

2.3 *ASHRAE Documents:*[4]

Cooling Towers. Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment
Codes and Standards. Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment
Water Treatment. Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment
Minimizing the Risk of Legionellosis Associated with Building Water Systems

2.4 *ASM Documents:*

Manual of Clinical Microbiology, Fifth Edition [5]
Manual of Environmental Microbiology [6]

2.5 *CDC Documents:*[7]

Guidelines for Prevention of Nosocomial Pneumonia
Hospital-Laboratory Diagnosis of Legionella Infections

[1] This guide is under the jurisdiction of ASTM Committee D22 on Sampling and Analysis of Atmospheres and 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, 1015 18th St. N.W., Washington, DC 20036, USA, 1990, 1989.

[4] Available from the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 1791 Tullie Circle, NE, Atlanta, GA 30329, USA.

[5] Winn, W.C., "*Legionella*," in *Manual of Clinical Microbiology*, Murray, P.R., Ed., American Society for Microbiology, Washington, DC 20005, USA, 1999, pp. 572–585.

[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, 1997, pp. 666–675.

[7] Available from the U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA, 1987, 1994, 1996, 1997, 2000.

Procedures for the Recovery of Legionella from the Environment

Final Recommendations to Minimize Transmission of Legionnaires' Disease from Whirlpool Spas on Cruise Ships

Case Definitions for Infectious Conditions Under Public Health Surveillance

Guidelines for Preventing Opportunistic Infections Among Hematopoietic Stem Cell Transplant Recipients

Occupational Safety and Health Administration Technical Manual, Section II - Chapter 7, Legionnaires' Disease

2.6 *State of Maryland Documents:*[8]

Report of the Maryland Scientific Working Group to Study Legionella in Water Systems in Healthcare Institutions

3. Terminology

3.1 Definitions from Compilation of ASTM Standard Definitions.

3.1.1 *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.

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

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

3.1.4 *sampling*, *n*—obtaining a representative portion of the material concerned.

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

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

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 *aerosol*, *n*—solid or liquid particles suspended in air.

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

3.2.4 *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.

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

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

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

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

3.2.9 *biocide*, *n*—*for legionellae*, a chemical used to kill legionellae and other microorganisms.

3.2.10 *biofilm*, *n*—a layer of microorganisms contained in a matrix that may form a slime on surfaces in contact with water.

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

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

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

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

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

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

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

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

3.2.19 *cooling tower*, *n*—a structure for lowering water temperature evaporatively by contact with atmospheric air.

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

3.2.21 *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.

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

3.2.23 *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.

3.2.24 *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.

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

3.2.26 *enzyme immunoassay (EIA)*, *n*—a technique to detect very small quantities of antigens through use of an anti-antibody attached to an enzyme that causes a color change in its substrate

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

[8] Available from State of Maryland, Department of Health & Mental Hygiene, Baltimore, MD, USA, 2000.

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

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

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

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

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

3.2.32 *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.

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

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

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

3.2.36 *IFA*, *adj*—indirect fluorescent-antibody.

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

3.2.38 *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*.

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

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

3.2.41 *indirect fluorescent-antibody test*, *n—for legionella antibodies*, a staining procedure that detects serum antibodies to legionellae through the use of bacteria fixed on a glass slide; secondary test antibodies labelled with fluorescent compounds attach to fixed legionellae/serum antibody complexes and fluoresce when viewed under appropriate irradiation.

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

3.2.43 *inspector*, *n*—a person examining an environment for possible contamination with legionellae.

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

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

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

3.2.47 *Legionella*, *n*—a bacterial genus containing over 40 species and at least 50 serogroups; abbreviated to the first initial when used repeatedly with species names, for example, *L. pneumophila*.

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

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

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

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

3.2.52 *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.

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

3.2.54 *microorganism*, *n*—a microscopic organism.

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

3.2.56 *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.

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

3.2.58 *PCR*, *adj*—polymerase chain reaction.

3.2.59 *polymerase chain reaction test*, *n*— a technique for selecting and amplifying specific genetic sequences.

3.2.60 *Pontiac fever*, *n*—a self-limited, short-duration, nonfatal disease characterized by fever and cough caused by or associated with legionellae.

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

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

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

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

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

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

3.2.67 *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.

3.2.68 *specificity*, *n— of a test for legionellosis or legionellae*, 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.

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

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

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

3.2.72 *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.

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

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

3.3 Refer to Terminology D 1129 and Terminology D 1356 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 legionellae; and (*3*) recognition and diagnosis of legionellosis. Section 7 describes environmental inspections of water systems for legionellae and suggests general control measures to limit legionella multiplication. Section 8 explains how to collect environmental samples to detect the presence of legionellae. 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 *inspectors* when referring to people examining the environment for possible legionella contamination (see Section 7) and the term *investigators* when referring to people conducting epidemiological studies of possible legionellosis outbreaks (see Section 9). An inspection or investigation team 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 legionellae 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 legionellae in the absence of illness (see 5.2) and for single legionellosis cases (see 5.3). Refer also to the CDC Guidelines for Prevention of Nosocomial Pneumonia and Guidelines for Preventing Opportunistic Infections Among Hematopoietic Stem Cell Transplant Recipients. Detecting legionellae in a water system is not sufficient to identify the system as a health hazard. However, failure to detect legionellae does not indicate, conclusively, that the bacteria are 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 isolating and identifying legionellae. Isolation of apparently identical legionellae from clinical and environmental samples (see 6.2.1, 6.6.2.1, 8) may suggest that a water system was the source of 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. 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 Legionellae 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 legionellae and may be able to implement control measures before the bacteria reach amounts sufficient to cause human illness (see 6.2.4.2). The CDC 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 legionellae in water samples from a center's potable water supply could be regarded as part of an overall strategy for preventing Legionnaires' disease in transplant centers. 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 legionellae in the absence of illness, particularly in buildings other than hospitals or other health-care facilities, given that absolute exclusion of these bacteria 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 legionellae in environmental samples may cause unwarranted alarm and unnecessary remediation.

5.3 *Environmental Testing for Legionellae 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 detecting legionellae. 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 People 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 legionella from clinical and environmental samples (see 6.2.1, 6.6.2.1, and Section 8) 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 an environmental source responsible for legionella transmission may be difficult if no clinical isolate is available for comparison with environmental isolates (see 6.2.1, 6.6.2.1). Legionellae have 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 other information (see Section 7).

5.4 *Environmental Testing for Legionellae 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 legionellae are detected or the potential source is implicated in patient exposure.

6. Background

6.1 *Legionellae*—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, and Refs **(1-3)** for background information on legionellae.

6.1.1 *The Genus Legionella*—Legionella are gram-negative, rod-shaped bacteria. Microbiologists currently recognize over 40 species in this genus of which at least 19 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 Legionellae—L. pneumophila* (in particular Serogroup 1, also Serogroups 4 and 6) accounts for more than 80 % of legionellosis cases that have been studied in the United States. Other species associated with clinical infections include *L. micdadei, L. dumoffii, L. bozemanii, L. feeleii*, and *L. longbeachae*.

6.1.3 *Legionellae in the Environment*— Legionellae are found world-wide 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). Legionellae live in biofilms near the surfaces of lakes, rivers, and streams and in conjunction with specific protozoa.

6.1.4 *Legionellae 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), water stagnation followed by agitation, and 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 bacteria do not survive in waters warmer than 60°C. Chlorinating potable water supplies may not eradicate legionellae (see 6.1.5). Low concentrations of legionellae (even below concentrations detectable by conventional test methods, see 6.2) can colonize water systems and can multiply under suitable conditions.

6.1.5 *Association of Legionellae with Other Organisms*—In humans, legionellae infect a type of white blood cell in the lungs whereas, in the environment, the bacteria infect free-living aquatic amebae and other protozoa (see 6.1.3 and 6.1.4). Legionellae inside protozoa may be protected from biocides, desiccation, and other environmental stresses.

6.2 *Microbiological Analysis of Environmental Samples for Legionellae*—Legionellae can be detected in environmental samples by three methods (*1*) growth of viable bacteria on culture medium (see 6.2.1); (*2*) detection of legionellae 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). The standard or primary laboratory method to detect legionellae is isolation (see 6.2.1). DFA and PCR results are available sooner than culture. All samples should be submitted for culture to determine bacterial viability and to obtain legionella isolates for serogroup and subgroup identification, as needed (see 6.2.1.2). Refer to Test Methods D 596, Practices D 2331, and Guide D 3856, the APHA Standard Methods for the Examination of Water and Wastewater, and the CDC Procedures for the Recovery of Legionella from the Environment for information on detection and identification of legionellae from environmental samples.

6.2.1 *Isolation of Legionellae from Environmental Samples*:

6.2.1.1 *Primary Isolation*—Water samples and washings of other materials (see Section 8) can be inoculated onto culture medium directly, after dilution, or after concentration by centrifugation or filtration. Samples may be treated with heat or buffered acid solution to reduce the numbers of nonlegionella organisms. The detection limit for culture methods typically is one colony-forming unit mL^{-1}. The specificity of legionella isolation from environmental samples is 100 %, but its sensitivity may vary depending on the 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 legionellae 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 (see 6.2.2 and 6.6.2.2) or other test. Laboratories should preserve (until completion of the investigation) environmental legionella isolates from outbreaks for possible further examination by public health authorities and for more specific identification by methods that may not be available commercially (see 5.3.2 and 6.6.2.1).

6.2.2 *Direct Fluorescent-Antibody (DFA) Test for Environmental Samples*—Microbiologists can detect bacteria in environmental samples with DFA stains similar to those used to identify culture isolates (see 6.2.1.2 and 6.6.2.1) and to detect legionellae directly in clinical specimens (see 6.6.2.2). However, DFA stains react with both living and dead legionellae and also may stain other bacteria, giving false-positive test results. Legionellae in water samples and washings of other materials (see Section 8) typically are concentrated by filtration or centrifugation before staining. The DFA detection limit for legionellae in water samples is 10 cells mL^{-1}. This method allows rapid sample screening because results are available in one day.

6.2.3 *Polymerase Chain Reaction (PCR) Test for Environmental Samples*—The PCR technique selects pre-determined sequences of genetic material and then amplifies and labels them with detectable markers. The PCR technique, although specific, amplifies genetic material from living and dead legionellae. Legionellae in water samples and washings of other materials (see Section 8) typically are concentrated by filtration or centrifugation before testing. Not all environmental samples can be analyzed by PCR because some samples may contain compounds or materials that interfere with or inhibit a PCR test. The PCR detection limit, in theory, is a single, intact copy of a target genetic sequence. Current PCR systems for legionella in water samples are designed to detect 10 to 100 cells mL^{-1}. This method allows rapid sample screening because results are available in one day, but kits to conduct the test are no longer commercially available.

6.2.4 *Interpreting Water Sampling Results*—Determine, before testing environmental samples for legionellae, (*1*) the reasons for sampling (see Section 5); (*2*) how to interpret laboratory results (see 6.2.4.1 and 6.2.4.2); and (*3*) what action to take based on the information obtained (see Section 10). Use only culture methods (see 6.2.1) to document legionella presence conclusively in environmental samples because the DFA test occasionally gives false-positive results (see 6.2.2) and the PCR procedure currently is experimental (see 6.2.3).

6.2.4.1 *Legionellae Not Detected*—Rule out the possibility of false-negative test results when legionellae are not detected in environmental samples before concluding the bacteria are not present. Possible reasons for not detecting legionellae that are present are (*1*) limited sample number or volume (see 8.2 and 8.3.1); (*2*) testing unconcentrated samples (see 6.2.1.1 and 6.2.2); (*3*) culturing samples without heat or acid treatment (see 6.2.1.1); (*4*) failing to run proper control samples to detect field or laboratory errors; (*5*) collection of unrepresentative samples; and (*6*) improper collection or handling of samples (see 8.3 and 8.4). Detection methods that rely on culturing legionellae (see 6.2.1) may fail to isolate them if the bacteria lose viability during sample storage or transport to a laboratory or during the culturing process, for example, as a result of heat or acid treatment (see 6.2.1.1). Laboratories also may fail to isolate legionellae by the culture method if the bacteria have lost viability due to biocide treatment or natural die-off or if the bacteria are unable to grow on available culture media or under given laboratory conditions.

6.2.4.2 *Legionellae Detected*—Detection of legionellae in environmental samples by the culture method (see 6.2.1) is not uncommon (see 6.1.3 and 6.1.4). Experts do not agree on the reliability of methods to quantify legionellae or on the concentrations of these bacteria in various water supplies that represent hazardous situations. Legionellae detected by DFA (see 6.2.2) or PCR (see 6.2.3) may be viable or non-viable by the culture method (see 6.2.1). Pontiac fever can result from exposure to non-viable legionellae (see 6.3, 6.5). However, only viable legionellae can cause Legionnaires' disease (see 6.3 and 6.4).

6.2.5 *Air Monitoring for Legionellae*— Investigators have isolated legionellae from air samples collected near sources associated with Legionnaires' disease outbreaks; for example, operating HVAC equipment before decontamination (**4**). However, do not rely on air sampling to measure potential exposure to legionellae because of the high likelihood of failure to detect the bacteria. Inspectors may obtain false-negative test results if the concentration of airborne legionellae is below an air sampling method's detection limit. Detection methods that rely on culturing legionellae (see 6.2.1) may fail to isolate them from air samples if the bacteria lose culturability while airborne, during the collection procedure, during sample storage or transport to a laboratory, or during the culturing process. Methods not based on bacterial multiplication (for example, DFA and PCR tests, see 6.2.2 and 6.2.3) may detect legionellae in air samples testing negative by the culture method.

6.3 *Legionellosis*—The term *legionellosis* is used for any disease caused by or associated with legionellae (see 6.1). Inhaling airborne legionellae and aspirating the bacteria into the lungs can lead to two types of disease, that is, Legionnaires' disease and Pontiac fever (see 6.4 and 6.5). Possible explanations for the manifestation of two disease syndromes caused by the same bacteria include the inability of some legionellae to multiply in human tissue (for a variety of reasons, including virulence, host range, or viability of the bacteria) and differences in host susceptibility. Exposure to the same environmental source has resulted in pneumonia and a nonpneumonic, Pontiac fever-like illness (**5**). Exposure to legionellae may occur indoors or outdoors, in residences, workplaces, or public settings, but infection is not transmitted from person to person. Legionnaires' disease may occur as isolated, sporadic cases or as outbreaks when several people are exposed to the same source and become infected (see 6.3.3). Pontiac fever, by definition, is an epidemic disease. Refer to the ASM Manual of