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

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^{ε1} NOTE—Paragraphs 1.1, 2.1, and 6.2 were editorially corrected in January 2001.

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 manmade 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 due to inhalation of airborne legionellae (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:

- D 512 Test Methods for Chloride Ion in Water²
- D 596 Practice for Reporting Results of Analysis of Water²
- D 887 Practices for Sampling Water-Formed Deposits³
- D 1067 Test Methods for Acidity or Alkalinity of Water²
- D 1129 Terminology Relating to Water²
- D 1192 Specification for Equipment for Sampling Water and Steam in Closed Conduits²
- D 1293 Test Methods for pH of Water²
- D 1356 Terminology Relating to Sampling and Analysis of Atmospheres⁴
- D 2331 Practices for Preparation and Preliminary Testing of Water-Formed Deposits³

D 3370 Practices for Sampling Water from Closed Conduits²

D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water²

D 4840 Guide for Sample Chain-of-Custody Procedures²

E 645 Test Method for Efficacy of Microbicides Used in Cooling Systems⁵

E 1427 Guide for Selecting Test Methods to Determine the Effectiveness of Antimicrobial Agents and Other Chemicals for the Prevention, Inactivation, and Removal of Biofilm⁵

2.2 APHA Documents:

The Public Health Law Manual, Second Edition⁶
Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition⁶

2.3 ASHRAE Documents:

Cooling Towers. Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment⁷

Codes and Standards. Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment⁷

Water Treatment. Handbook—Heating, Ventilating, and Air-Conditioning Systems and Equipment⁷

2.4 ASM Documents:

Manual of Clinical Microbiology, Fifth Edition⁸

2.5 CDC Documents:

Guideline for Prevention of Nosocomial Pneumonia⁹
Hospital-Laboratory Diagnosis of Legionella Infections⁹
Procedures for the Recovery of Legionella from the Environment⁹

⁵ Annual Book of ASTM Standards, Vol 11.05.

⁶ Available from the American Public Health Association, 1015 18th St. N.W., Washington, DC 20036, USA, 1990, 1989.

⁷ Available from the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 1791 Tullie Circle, NE, Atlanta, GA 30329, USA.

⁸ Rodgers, F. G., and Pasculle, W., "Legionella," in *Manual of Clinical Microbiology*, Balows, A., Ed., American Society for Microbiology, Washington, DC 20005, USA, 1991, pp. 442-453.

⁹ 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, 1992, 1994.

¹ This guide is under the jurisdiction of ASTM Committee D-22 on Sampling and Analysis of Atmospheres and is the direct responsibility of Subcommittee D22.05 on Indoor Air.

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² Annual Book of ASTM Standards, Vol 11.01.

³ Annual Book of ASTM Standards, Vol 11.02.

⁴ Annual Book of ASTM Standards, Vol 11.03.

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

laboratory test as caused by or associated with legionella infection.

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

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

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

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

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

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

3.2.31 *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.

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

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

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

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

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



3.2.37 *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*.

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

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

3.2.40 *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.

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

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

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

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

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

3.2.46 *Legionella*, *n*—a bacterial genus containing at least 30 species and at least 50 serogroups; abbreviated to the first initial when used repeatedly with species names, for example, *L. pneumophila*.

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

3.2.48 *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.

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

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

3.2.51 *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.

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

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

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

3.2.55 *outbreak*, *n*—*of legionellosis*, the occurrence of two or more confirmed or probable 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 kilometers around a potential source); case

occurrence exceeding the number expected in a given time period and locale.

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

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

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

3.2.59 *Pontiac fever*, *n*—a self-limited, short-duration, non-fatal fever caused by or associated with legionellae.

3.2.60 *probable case*, *n*— *of legionellosis*, the occurrence of an illness clinically compatible with legionellosis in a person with a legionella antibody titer of 256 or higher when only a single blood sample is available.

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

3.2.62 *RIA*, *adj*—radioimmunoassay.

3.2.63 *radioimmunoassay test*, *n*—an immunological procedure in which a radioisotope-labelled compound is reacted with a test material and the attached radioisotope is detected by various means.

3.2.64 *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.

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

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

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

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

3.2.69 *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.

3.2.70 *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.

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

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

3.2.73 *surveillance*, *n*— *of legionellosis*, the supervision or inspection of aspects of legionellosis occurrence pertinent to effective control.

3.2.74 *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.

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

3.2.76 *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. 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). 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 iden-

tify undesirable situations by monitoring routinely for legionellae and may be able to implement control measures before the bacteria reach concentrations sufficient to cause human illness (see 6.2.4.2).

5.2.2 Some experts advise against testing water systems for legionellae in the absence of illness 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 expensive 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 identification 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, 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) because legionellae have been found in a substantial proportion of water systems tested in prevalence surveys and outbreak investigations.

5.4 *Environmental Testing for Legionellae for Multiple Legionellosis Cases*—Identification of multiple, suspected or confirmed, legionellosis cases 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 other legionellosis patients so they receive appropriate care (see 6.4.4, 6.5.4). 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, and Refs (1-4) for background information on legionellae.

6.1.1 *The Genus Legionella*—Legionella are gram-negative, rod-shaped bacteria. Microbiologists currently recognize at least 35 species in this genus of which at least 17 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. 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 algae and protozoa. 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 (see 6.1.4).

6.1.4 *Legionellae in Man-Made Water Systems*—Factors known to enhance legionella colonization of man-made water systems (see 6.1.3, 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.

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 may infect some free-living aquatic amoebae 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, that is, isolation of the bacteria (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 section 6.2.1.3). 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, 6.6.2.2) or other test. Laboratories should preserve environmental legionella isolates from outbreak investigations 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, 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 section 6.2.1.3, 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.

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, 6.2.4.3); 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; (2) testing unconcentrated samples; (3) culturing samples without heat or acid treatment (see 6.2.1.1); and (4) failing to run proper control samples to detect field or laboratory errors. 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 unacceptable hazards. 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 (5). 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 possibly 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). Infectious disease experts do not understand why one group of organisms produces two distinct illnesses with different attack

rates and severities (see 6.4.5, 6.4.7, 6.5.5, 6.5.7). 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 and Pontiac fever 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). Refer to the ASM Manual of Clinical Microbiology, the CDC Guideline for Prevention of Nosocomial Pneumonia, and (4) for background information on legionellosis.

6.3.1 *History of Legionellosis*—In 1977, the U.S. Centers for Disease Control and Prevention (CDC) identified a bacterium as the causative agent of a pneumonia outbreak at a 1976 American Legion Convention in Philadelphia. This bacterium later was named *Legionella pneumophila*. The 1976 outbreak resulted in more than 200 Legionnaires' disease cases and 34 deaths among the more than 4000 convention attendants. Although legionellae undoubtedly caused disease before 1976 (at least two prior Legionnaires' disease outbreaks have been identified), laboratories failed to isolate or detect the bacteria because of their unusual growth requirements and poor staining characteristics (see 6.1).

6.3.2 *Incidence of Legionellosis*—Legionnaires' disease is a serious but fairly common form of pneumonia (see 6.4) responsible for an estimated 3 to 15 % of adult hospitalizations for community-acquired pneumonia. Researchers have estimated that, annually in the United States, between 15 000 and 75 000 people with Legionnaires' disease require hospitalization. The actual incidence of Legionnaires' disease probably is substantially higher than the number of reported cases (see 9.2) because some patients do not require hospitalization and it is not possible to distinguish this infection from other forms of pneumonia without confirmatory laboratory tests (see 6.4, 6.6). The incidence of Pontiac fever is not known (see 9.2) but it appears to be fairly common.

6.3.3 *Legionellosis Outbreaks and Sporadic Cases*—A legionellosis outbreak is defined as (1) the occurrence of two or more cases linked by time of onset and location; or (2) the occurrence of cases exceeding the number expected in a given time period and locale based on previously observed incidence of the disease. At least 65 to 80 % of Legionnaires' disease cases reported in the United States and the United Kingdom apparently occur as sporadic cases, that is, isolated events in which no other cases are identified (see 9.1, 9.3.3). Underreporting of sporadic legionellosis cases probably is even higher than underreporting of cases that occur in clusters (see 6.3.2, 9.2). Legionellae may cause a large percentage of sporadic hospital-acquired pneumonias (see 6.4.5).

6.3.4 *Sources Implicated in Legionellosis Outbreaks*—Legionellosis outbreaks have been associated with exposure to contaminated aerosols generated by cooling towers, evaporative condensers, spas, respiratory therapy and dental equipment, showers, water faucets, decorative fountains, ultrasonic mist machines, and damp potting soil.

6.3.5 *Legionella Transmission*—The likelihood of legionella transmission and subsequent infection is related to (1) the concentration of legionella in a water system; (2) spraying or splashing of contaminated water and transfer of legionellae

to the air; (3) air temperature and moisture content; (4) the presence of amoebae and other protozoa that may protect legionellae; (5) the intensity and duration of a person's exposure to airborne legionellae; and (6) an exposed person's susceptibility (see 6.4.2, 6.5.2).

6.4 *Clinical Aspects of Legionnaires' Disease*—Refer to the ASM Manual of Clinical Microbiology, the CDC Guideline for Prevention of Nosocomial Pneumonia, and (4) for information on clinical aspects of Legionnaires' disease.

6.4.1 *Symptoms*—Legionnaires' disease is a form of pneumonia with other multiple-system signs and symptoms. Symptoms may range from mild cough and low fever to rapidly progressive pneumonia and coma. Early symptoms include malaise, muscle pain, and headache; later symptoms include high fever (40.5°C), unproductive dry cough, and shortness of breath. Legionnaires' disease patients may report gastrointestinal symptoms including vomiting, diarrhea, nausea, and abdominal pain.

6.4.2 *Risk Factors*—Legionnaires' disease is usually an opportunistic infection occurring most often in the elderly and in people who smoke cigarettes, consume alcohol, have chronic cardiovascular or pulmonary conditions, or are immunocompromised. People may be immunocompromised due to illness (for example, cancer or viral infection) or medical treatment (for example, radiation therapy or medication). Medications that may increase a person's susceptibility to Legionnaires' disease are those that suppress the immune system, including prolonged use of steroids, many cancer chemotherapy treatments, and medications used with organ transplant.

6.4.3 *Incubation Period*—The incubation period for Legionnaires' disease is generally two to ten days.

6.4.4 *Treatment*—Antibiotic treatment of Legionnaires' disease reduces mortality. Erythromycin is the drug of choice, and rifampin is sometimes used in severe cases. Newer antibiotics related to erythromycin, such as azithromycin, also appear to be effective.

6.4.5 *Attack Rate*—Usually fewer than 5 % of people exposed in community-acquired Legionnaires' disease outbreaks become ill. The attack rate may be higher in outbreaks of hospital-acquired Legionnaires' disease.

6.4.6 *Sequelae*—Recovering Legionnaires' disease patients may continue to suffer fatigue and respiratory symptoms for several months.

6.4.7 *Mortality*—Ten to 15 % of people with community-acquired Legionnaires' disease die due to progressive pneumonia and shock. The fatality rate for hospital-acquired legionellosis may be as high as 30 to 50 %.

6.5 *Clinical Aspects of Pontiac Fever*—The exact role legionellae play in Pontiac fever is not clear. Legionellae have never been isolated (see 6.6.2.1) from clinical specimens of people with Pontiac fever. The association between Pontiac fever and legionella is based on serological evidence (see 6.7) and on a history of exposure to a potential legionella source (see 6.3.4). Refer to the ASM Manual of Clinical Microbiology, the CDC Guideline for Prevention of Nosocomial Pneumonia, and (4) for information on clinical aspects of Pontiac fever.

6.5.1 *Symptoms*—Pontiac fever is a self-limited, short-duration, non-fatal fever. Symptoms include chills, headache, muscle pain, and other influenza-like complaints.

6.5.2 *Risk Factors*—Pontiac fever often affects otherwise healthy people without underlying medical conditions.

6.5.3 *Incubation Period*—The period between exposure and symptom onset in Pontiac fever is generally 6 to 48 h.

6.5.4 *Treatment*—People with Pontiac fever recover completely in two to five days without medical intervention.

6.5.5 *Attack Rate*—The attack rate in Pontiac fever outbreaks may be as high as 95 %.

6.5.6 *Sequelae*—Recovery from Pontiac fever is complete without further complications or complaints.

6.5.7 *Mortality*—Death has not occurred due to Pontiac fever.

6.6 *Diagnosing Legionnaires' Disease*—Refer to the ASM Manual of Clinical Microbiology, the CDC Hospital-Laboratory Diagnosis of Legionella Infections and Guideline for Prevention of Nosocomial Pneumonia, and (4) for a discussion of the diagnosis of Legionnaires' disease.

6.6.1 *Case Definition*—The CDC's surveillance case definition for Legionnaires' disease requires (1) pneumonia documented by chest x-ray; and (2) at least one positive clinical laboratory test (see 6.6.2). Laboratory tests are necessary to confirm a diagnosis of Legionnaires' disease because the symptoms and x-ray patterns of this form of pneumonia are not unique.

6.6.2 *Confirmatory Clinical Laboratory Tests*—A diagnosis of Legionnaires' disease can be confirmed, using the current CDC case definition (see 6.6.1), by any one of the following laboratory findings (1) isolation of legionellae from clinical specimens (see 6.6.2.1); (2) detection, with a direct fluorescent-antibody (DFA) stain, of legionellae in clinical specimens (see 6.6.2.2); (3) detection, with a radioimmunoassay (RIA) test, of *L. pneumophila* Serogroup 1 antigens in urine (see 6.6.2.3); or (4) a four-fold or greater rise in *L. pneumophila* antibody titer measured with an indirect fluorescent-antibody (IFA) test (see 6.6.2.4). It also may be possible to detect legionellae in clinical specimens using the polymerase chain reaction (PCR) test. Do not use unproven diagnostic tests (for example, enzyme-linked immunosorbent assays) to confirm a diagnosis of Legionnaires' disease.

6.6.2.1 *Isolation of Legionellae from Clinical Specimens*—The most definitive test to confirm the presence of legionellae in a patient is the isolation of viable bacteria from sputum, bronchial brush or washing, transtracheal aspirate, or other clinical or autopsy specimen. The specificity of legionella isolation from clinical samples is 100 % and its sensitivity is approximately 70 % (see 6.6.2.5). Collect samples before patients begin antibiotic treatment, if possible. The specific species, serogroup, and subtype (see 6.1.1 and 6.1.2) to which a clinical legionella isolate belongs may be identified with a DFA (see 6.6.2.2) or other test. Preserve clinical legionella isolates for possible further examination by public health authorities and for more specific identification by methods that may not be available commercially (see Section 5, 5.3.2, 6.2.1.3).

6.6.2.2 *Direct Fluorescent-Antibody (DFA) Test on Clinical*

Specimens—Laboratories may detect legionellae, with a DFA stain, directly in lung aspirates or tissue sections. The specificity of a controlled and carefully performed clinical DFA test approaches 100 % and its sensitivity is approximately 75 % (see 6.6.2.5). A DFA test may give false-negative results early in the disease process when few organisms are present or if the test reagent does not include antibodies specific to the legionella causing a patient's infection. False-positive cross reactions with nonlegionella organisms also have been reported.

6.6.2.3 Urine Antigen Test—Laboratories can detect legionella antigens, with a radioimmunoassay (RIA) test, in the urine of active and recently recovered Legionnaires' disease patients. The test detects antigens on bacterial cells the body passes into the urine during the disease process and for as long as several months thereafter. Use of a urine antigen test to detect Legionnaires' disease is limited, at present, by the commercial availability of reagents for only *L. pneumophila* Serogroup 1 (see 6.6.2.5).

6.6.2.4 Legionella Antibody Titer in Blood—An indirect fluorescent-antibody (IFA) test detects legionella antibodies in blood serum. The specificity of a controlled and carefully performed IFA test approaches 100 % and the sensitivity is approximately 75 % (see 6.6.2.5). Laboratories report serum antibody titer as the reciprocal of the highest two-fold dilution showing a positive reaction. For example, a titer of 256 would show positive reactions at dilutions of 1/64, 1/128, and 1/256, but not at 1/512. A four-fold or greater rise in *L. pneumophila* antibody titer to at least 128 in a blood sample collected in the convalescent phase of a patient's illness as compared to an acute-phase sample demonstrates recent infection. Store sera until one technician can test paired acute- and convalescent-phase samples on the same day using the same reagents. A single legionella antibody titer of 256 or higher is considered suggestive of legionella infection (see 6.6.3).

6.6.2.5 Precautions on Diagnosing Legionnaires' Disease—No laboratory test for Legionnaires' disease diagnosis is 100 % sensitive, that is, infection is not ruled out even if one or more of the above tests are negative. The earlier in the course of an illness a culture, DFA stain, or urine antigen test is performed the better the chances of detecting Legionnaires' disease. A single serological test is less useful in the first weeks of acute Legionnaires' disease than three to six weeks after symptom onset when the infected person has produced detectable levels of legionella antibodies (see 6.6.2.4, 6.6.3).

6.6.3 Probable Legionnaires' Disease Cases—A probable, but unconfirmable, case of Legionnaires' disease is defined as an illness with fever, pneumonia, and a convalescent legionella antibody titer of 256 or higher (see 6.6.2.4). Studies have found legionella titers of 256 or higher in 1 to 20 % of healthy adults, typically approximately 5 %. Therefore, a single elevated antibody titer does not confirm Legionnaires' disease (see 6.6.2.4) but when seen with a compatible illness defines a probable case during an epidemiological investigation (see Section 9).

6.7 Diagnosing Pontiac Fever—Refer to the ASM Manual of Clinical Microbiology, the CDC Hospital-Laboratory Diagnosis of Legionella Infections, and (4) for a discussion of the

diagnosis of Pontiac fever. Pontiac fever is diagnosed, in association with a flu-like illness (see 6.5), by an antibody titer of 256 or higher (see 6.6.2.4, 6.6.3) to *L. pneumophila* or to an environmental legionella isolate from a source to which the patient was exposed (see 6.1.3, 6.1.4, 6.2.1, 6.3.4, 8.2).

7. Procedure—Environmental Inspections of Water Systems to Identify Potential Legionella Sources, and General Measures to Control Legionella

7.1 This section outlines an inspection considered appropriate (1) for water systems associated with multiple legionellosis cases; and (2) periodically (for example, every one, five, or ten years) for other systems. Factors important in preventing situations that may lead to legionella transmission include (1) understanding of the environmental conditions that support legionella multiplication (see 6.1.4); and (2) awareness of the types of water systems and equipment that may harbor legionellae and may generate aerosols (see 7.3.1-7.3.6). The purpose of a water system inspection may be (1) to identify and examine water systems in which legionella could multiply and from which the bacteria could become airborne; and (2) to suggest control measures to correct observed and potential problems. Refer to the ASHRAE Codes and Standards, Cooling Towers, and Water Treatment and Refs (1-4) for information on environmental inspections of water systems for legionellae and on general control measures.

7.2 Gathering Preliminary Information on Water System Design, Operation, and Maintenance:

7.2.1 System Design—Review up-to-date blueprints or schematic drawings of facility water and ventilation systems. Use *as built* plans if systems differ from their original designs.

7.2.2 System Operation and Maintenance—Examine operation and maintenance records for all water systems including hot water supplies and water-cooled heat-transfer equipment (see 10.2.5). Review records of water temperature and biocide concentration measurements, of dates and types of water treatment, and of dates and results of visual inspections and water quality testing. Inquire about recent major maintenance on water systems or changes in their operation or use.

7.3 Walkthrough Visit—Ask a facility engineer or maintenance staff member familiar with the water system to assist during walkthrough visits. Inspect hot and cold water systems including heaters, chillers, storage tanks, distribution piping, water treatment equipment, connections protected by back-flow preventers, and the like (see 7.3.1.2). Carry a thermometer, flashlight, note paper, and camera or video recorder on walkthrough visits. Request that equipment be turned off while examining it, if possible. Wear disposable garments, slip-proof footwear, and eye protection while examining areas that are wet, potentially contaminated, or recently treated with biocides, disinfectants, detergents, or other chemicals. Wear at least a 95 % efficient half-mask respirator when working near potentially contaminated equipment that might generate aerosols.

7.3.1 General Water Supply:

7.3.1.1 Water Stagnation—Identify portions of systems in which water may stagnate, for example, storage tanks, unused plumbing sections, and faucets operated less often than monthly (see 10.3.5, 10.4.6).



7.3.1.2 *Connections Between Potable and Non-Potable Water Systems*—Look for connections between potable water supplies and waters used for cooling and for supplying fire sprinklers and other devices (see 10.4.5). Examine the condition and types of devices used to prevent back flow at these connections. Ask if the facility experienced a water-pressure loss, for example, due to line breakage or street repairs, because failure of a back-flow preventer during a pressure loss can contaminate a water supply.

7.3.1.3 *Hot and Cold Water Line Separation*— See if hot and cold water lines are separated physically or if hot water lines are insulated to prevent heat transfer (see 10.3.3, 10.4.3).

7.3.2 *Hot Water Supply:*

7.3.2.1 *Hot Water Holding Temperature*— Measure and record initial and final equilibrium water temperature in each storage unit fed by a hot water heater. It may be necessary to run water through a drain or outlet port for several minutes before water temperature stabilizes. Store hot water at or above 60°C (see 7.4, 10.3.2) to limit legionella multiplication. Do not rely on the reading of a water heater's temperature gage, because the thermometer may not be accurate and heat stratification may result in unrepresentative readings.

7.3.2.2 *Hot Water Delivery Temperature*— Measure and record water temperature in hot water lines throughout a facility, for example, at faucets nearest, intermediate, and most distant from the hot water heater or storage tank. Record initial and final equilibrium water temperatures in hot water supply lines. It may be necessary to run a faucet for several minutes before water temperature reaches its maximum at distant locations in a system. Deliver hot water at a temperature of 50°C or higher, if permitted (see 7.4, 10.3.2).

7.3.2.3 *Hot Water Sample Appearance*—Note the presence of rust, scale, and other material in samples drawn to measure hot water temperature (see 7.3.2.1 and 7.3.2.2), which may indicate infrequent use, corrosion, or biofilm formation.

7.3.3 *Cold Water Supply:*

7.3.3.1 *Cold Water Storage Temperature*— Measure and record the temperature of water drawn from each cold water storage unit. Store cold water at or below 20°C (see 7.4, 10.4.2) to limit legionella multiplication. Examine storage tanks for cold water systems used as reserve capacity or to maintain hydrostatic pressure. Protect these systems from temperatures below 0°C and above 20°C and cover them to prevent contamination with organic debris and organisms that may support legionella multiplication.

7.3.3.2 *Cold Water Delivery Temperature*— Measure and record water temperature in cold water lines throughout a facility, for example, at faucets nearest, intermediate, and most distant from the main cold water source or storage tank. Record initial and final equilibrium water temperature in cold water supply lines. It may be necessary to run a faucet for several minutes before water temperature reaches its minimum at distant locations in a system. Protect cold water lines from heat sources to limit legionella multiplication.

7.3.3.3 *Cold Water Sample Appearance*—Note the presence of rust, scale, and other material in samples drawn to measure cold water temperature (see 7.3.3.1 and 7.3.3.2), which may indicate infrequent use, corrosion, or biofilm formation.

7.3.4 *Heating, Ventilating, and Air-Conditioning (HVAC) Systems*—Examine humidifiers, cooling towers, evaporative condensers, air washers, and similar equipment (see 7.3.5, 10.5). Note the locations of outdoor air intakes for HVAC systems relative to aerosol sources such as the air exhausts for water-cooled heat-transfer systems (see 7.3.5.2, 10.2.1.3, 10.5.1).

7.3.5 *Water-Cooled Heat-Transfer Systems:*

7.3.5.1 *Visual Evaluation*—Examine cooling towers, evaporative condensers, and similar water-cooled heat-transfer equipment for visible evidence of algal growth, biofilm, rust, scale, and other signs of contamination or poor maintenance. Examine the general physical and mechanical condition of water-cooled heat-transfer equipment and determine the presence and condition of drift eliminators (see 10.2.1.2).

7.3.5.2 *Air Supplies and Exhausts for Water-Cooled Heat-Transfer Systems*—Examine the air supplies for heat-transfer units and the proximity to them of sources that could supply nutrients for legionella multiplication (see 7.3.5.3). Observe the location of air exhausts for water-cooled heat-transfer systems relative to outdoor air intakes for HVAC systems (see 7.3.4, 10.2.1.3, 10.5.1).

7.3.5.3 *Construction and Excavation Operations and Other Sources of Organic Materials*—Look for construction or excavation operations that could generate dust or plant or animal debris that when washed into a cooling system's water could supply nutrients to support legionella multiplication (see 7.3.5.2).

7.3.5.4 *Water Sumps*—Evaluate the condition of sumps that collect water from cooling towers, evaporative condensers, and similar equipment. Measure water temperature for systems in use at the time of a walkthrough visit.

7.3.6 *Miscellaneous Water Systems*—Examine miscellaneous water sources such as decorative fountains, tepid-water eye washes, safety showers, produce (that is, fruit and vegetable) misters, spray irrigation systems for lawns and plants, cooling waters for industrial purposes, spray-cooled cutting machines, molding presses, pasteurizers, roof sprays for humidity control and cooling, storage tanks for fire-sprinkler systems, and spas, whirlpool baths, and jacuzzis, (see 10.6 and 10.7).

7.4 *Assessing Results of Preliminary Inspections and Recommending Control Measures*—Use the results of the reviews described in 7.2 and 7.3 to decide if further action is needed to reduce the risks of legionella multiplication in a water system and of human exposure to airborne legionella. Measures to control legionellae in the environment include correcting improper design, operation, or maintenance of water supplies and water-cooled heat-transfer equipment (see Section 10) and maintaining proper hot and cold water temperatures.

8. Procedure—Environmental Sampling for Legionellae

8.1 This section describes collection of environmental samples during or following water system inspections to identify possible legionella sources (see Section 7). Contact a qualified laboratory to obtain clean, new, or sterilized containers and to arrange for sample transport and analysis. Refer to Practice D 887, Specification D 1192, Practices D 3370 and D 4840, Guide D 3856, and the APHA Standard Methods for

the Examination of Water and Wastewater for information on environmental sampling for legionellae.

8.2 *Environmental Sampling Sites*—Estimate the number of samples to be collected based on a facility's size and the number of water systems identified as potential legionella sources (see Section 7). Consider collecting samples from the following environmental sources (1) incoming water supplies; (2) water storage tanks and hot water heaters; (3) hot and cold water faucets and shower heads; (4) water-cooled heat-transfer equipment; and (5) humidifiers, spas, decorative fountains, and other water systems (see 7.3.6) suspected of harboring legionellae or linked epidemiologically with legionellosis patients (see 9.3.1-9.3.3).

8.2.1 *Water Storage Tanks and Hot Water Heaters*—Aseptically collect water samples from the bottom drains and outlet pipes of water storage tanks and hot water heaters.

8.2.2 *Water Faucets and Shower Heads*—Aseptically collect water samples from hot and cold water faucets and shower heads throughout a facility, for example, at faucets nearest, intermediate, and most distant from water heaters, storage tanks, and connections with municipal water supplies. Collect the first water that leaves an outlet after opening the tap and another sample after water temperature stabilizes (see 7.3.2.2, 7.3.3.2). Use sterile swabs to sample faucets and shower heads, and transport swabs submerged in sample water.

8.2.3 *Water-Cooled Heat-Transfer Equipment*—Sample the make-up water supply for water-cooled heat-transfer systems and for associated storage tanks, sumps, and reservoirs. Collect additional water samples at locations distant from the make-up water outlet and where water enters sprayers or misters, if possible. Include samples of sludge, sediment, and biofilm.

8.2.4 *Humidifiers, Spas, Decorative Fountains, and Other Equipment*—Collect water from tanks and reservoirs of humidifiers, spas, decorative fountains, and other equipment and from their supply waters for comparison. Include samples of sludge, sediment, and biofilm.

8.3 *Environmental Sample Collection:*

8.3.1 *Sample Volume*—Collect at least 10 to 100 mL of water from cooling towers and other water-cooled heat-transfer systems which may contain elevated concentrations of legionellae. Collect more than 1 L of potable waters which may contain lower concentrations of legionellae. Use clean, new, or sterilized containers to collect samples.

8.3.2 *Sample Description*—Measure water temperature immediately before or after sample collection. Wipe thermometers with 70 % alcohol and air dry, or use other appropriate disinfectant, between samples. Note the presence of rust, scale, and other material in samples (see 7.3.2.3, 7.3.3.3, 8.3.5).

8.3.3 *Filling Sample Containers*—Keep collection containers closed until ready to fill and reclose them immediately after sample collection. Collect separately the first water leaving a faucet or drainage port and later flushes (see 8.2.2). Rinse collection containers several times with sample water before filling, when not collecting a first flush, and when the containers do not contain a dechlorinating agent (see 8.3.4), to condition the containers and remove possible interfering compounds. Leave ample air space (2 to 3 cm) in containers to

facilitate mixing and resuspension of settled material at the laboratory.

8.3.4 *Stopping Residual Biocide Action*—Sodium thiosulfate may be added at the time of sample collection to neutralize residual chlorine and to prevent continued biocidal action during sample transport and storage. Refer to the APHA Standard Methods for the Examination of Water and Wastewater for further information. Neutralization of low chlorine concentrations, for example, those typically found in potable water supplies, may not be necessary as legionellae are fairly resistant to chlorine (see 6.1.3, 6.1.5) and unrestricted growth of other microorganisms may interfere with legionella isolation.

8.3.5 *Sample Identification*—Record the following for each sample (1) facility name; (2) initials of person collecting sample; (3) sampling site description; (4) sampling date and time (5) sample identification number; and (6) sample temperature, volume, and appearance. Document all custody transfers and storage conditions from the time of sample collection to final disposition. Refer also to Practice D 4840.

8.4 *Environmental Sample Transport and Storage*—Let samples reach ambient temperature and protect them from extreme temperatures during transport and storage, for example, temperatures below 3°C and above 30°C. Seal the necks of collection containers with tape, wrap them in absorbent paper, and place them in individual plastic bags if not delivered by hand to a testing laboratory. Samples should reach the laboratory within 24 h of collection. Hold samples at room temperature at the testing laboratory and process them within 24 h of receipt.

8.5 *Personal Protection During Environmental Sample Collection*—Request that equipment be turned off while collecting samples, if possible. Wear disposable garments, slip-proof footwear, and eye protection while working in areas that are wet, potentially contaminated, or recently treated with biocides, disinfectants, detergents, or other chemicals. Wear at least a 95 % efficient, half-mask respirator when working near potentially contaminated equipment that might generate aerosols.

9. Procedure—Epidemiological Investigations of Possible Legionellosis Outbreaks

9.1 Factors important to recognizing a legionellosis outbreak rapidly, to identifying its cause, and to initiating appropriate interventions are (1) understanding of the types of exposures that can result in legionellosis (see 6.3.4 and 6.3.5); (2) accurate recognition and diagnosis of legionellosis when it occurs (see 6.4-6.7); and (3) prompt action on the part of physicians, public health authorities, employers, facility owners and operators, and others when notified of such illness. Reasons to investigate possible legionellosis outbreaks include (1) prevention of further exposures; (2) initiation of procedures to identify other people with legionellosis so they receive appropriate diagnosis and treatment; and (3) assessment of the likelihood that people who contracted legionellosis within 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 kilometers around a potential source) shared a common exposure.

9.2 Identifying Possible Legionellosis Outbreaks— Reporting of suspected or confirmed Legionnaires' disease to local health authorities, for example, city or county health officers or state epidemiologists, is mandatory in most states in the United States (Fig. 1, Step 1.) Clinical laboratories also must report findings of Legionnaires' disease to local health authorities. Reporting of single Pontiac fever cases (see 6.5, 6.7) is not mandatory, but physicians and laboratories should alert local health authorities of apparent case clusters. Test people suspected of having legionellosis as described in 6.6 and 6.7 (Fig. 1, Step 2). Refer to the APHA Public Health Law Manual and to relevant local laws and codes for information on reporting the suspicion or diagnosis of legionellosis. Public health authorities, employers, and employees may request assistance from the federal or state Occupational Safety and Health Administration, if exposure may have occurred at a workplace.

9.3 Determining if Legionellosis Occurred in the Past:

9.3.1 Identifying Possible Exposures— An epidemiologist or trained nurse or physician should question people with legionellosis (see 9.2) regarding possible exposures to potential legionella sources (see 6.3.4). Obtain an exposure history from a patient's health-care provider or from a reliable family member, co-worker, or colleague, if a patient cannot respond directly.

9.3.2 Determining When and Where Exposure May Have Occurred— Exposure histories (see 9.3.1) should cover the period immediately preceding symptom onset, that is, the three-week period before onset of Legionnaires' disease symptoms (see 6.4.3) and the week before onset of Pontiac fever symptoms (see 6.5.3).

9.3.3 Searching for Other People Who Had Legionellosis— Public health authorities should search for legionellosis (1) at the residences and workplaces of legionellosis patients; (2) among other people with whom patients may have shared an

exposure, for example, classmates, social contacts, and other visitors to facilities patients frequented; and (3) in the surrounding community. Interview contacts ill for two or more days, and look for clustering of legionellosis-like illness in time or space. Use standardized questionnaires to identify people who may have had legionellosis (see Appendix X1) and to interview the health-care providers of patients seen for illnesses compatible with legionellosis (see Appendix X2).

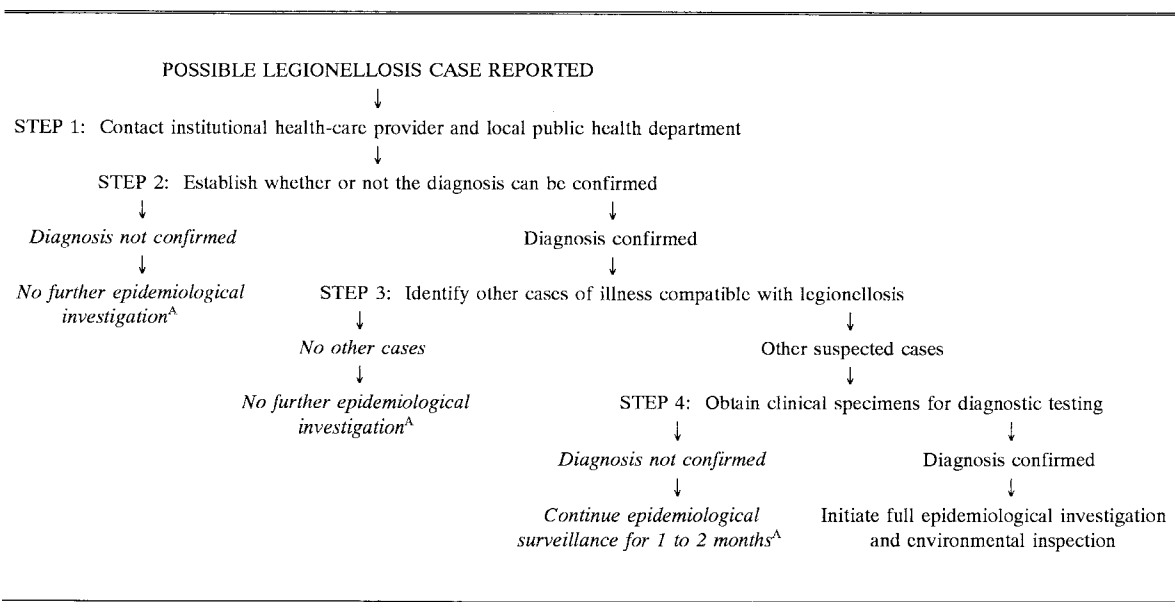
9.3.4 Confirming Suspected Legionellosis— Public health authorities should attempt to confirm apparent legionellosis by collecting and testing appropriate clinical specimens (Fig. 1, Step 3). Advise people possibly exposed to *L. pneumophila* Serogroup 1 to submit urine specimens for antigen testing (see 6.6.2.3). Collect both acute and convalescent blood samples from suspected patients, if possible, to detect a rise in legionella antibody titer (see 6.6.2.4). Less ideally, collect a single convalescent blood sample. Testing people with no history of legionellosis-like illness generally is not warranted (see 6.6.2.5, 6.6.3).

9.4 Determining if Legionellosis Continues to Occur:

9.4.1 Surveillance for New Legionellosis Cases— Public health authorities should inform local health-care providers of legionellosis outbreaks so physicians seeing patients with compatible illness will perform the necessary confirmatory tests and provide appropriate treatment. An epidemiologist or trained nurse or physician should interview people suspected of having legionellosis (see 6.4.1 and 6.5.1) and look for clustering of illness in time or space (see 9.3).

9.4.2 Surveillance Period— Continue surveillance for new legionellosis cases (see 9.4.1) for one to two months after initiation of an investigation or after investigation of the last suspected case (see Section 11 and Fig. 1, Step 4).

9.4.3 Confirming Legionellosis in Suspected New Cases— Attempt to confirm apparent legionellosis in suspect patients



^A Termination of an epidemiological investigation does not preclude initiation or continuation of an environmental inspection for possible sources of legionella multiplication or of exposure to legionellae.

FIG. 1 Flowchart for Epidemiological Investigation of Possible Legionellosis Outbreak

by testing appropriate clinical specimens (see 6.6 and 6.7, and Fig. 1, Step 4).

9.5 Action Needed if an Epidemiological Investigation Identifies Additional Legionellosis Cases—Initiate a full epidemiological investigation and environmental inspection of possible legionella sources if the search outlined in 9.3 or 9.4 identifies additional people with legionellosis (see 5.4). Initiate immediate control measures (see Section 10) on water systems identified as possible sources of legionella exposure if a legionellosis outbreak appears to be ongoing. If an investigation uncovers additional cases only retrospectively and no infections occurred since study initiation, investigators may delay implementing control measures for suspected legionella sources until environmental test results (see Sections 7 and 8) are available.

10. Procedure—Control Measures for Water Systems

10.1 The goal of control programs for water systems is to maintain adequately clean equipment and to avoid conditions that allow legionellae to multiply. The total eradication of these bacteria from water systems may not be possible or even necessary except in certain settings, for example, some health-care environments. Refer to the ASHRAE Codes and Standards and Water Treatment, the CDC Guideline for Prevention of Nosocomial Pneumonia, and (1-4) for information on general control measures for water systems.

10.2 Control Measures for Water-Cooled Heat-Transfer Systems:

10.2.1 Equipment Design and Location—Select designs and locate water-cooled heat-transfer equipment to facilitate water temperature control and prevent accumulation of organic material.

10.2.1.1 Construction Materials—Use durable, biocide-resistant materials for wet surfaces in water-cooled heat-transfer systems. Ensure that equipment will be accessible and will be easy to drain and to clean. See that air flow through each system is uniform and protect wetted surfaces from sunlight to minimize algal growth.

10.2.1.2 Drift Eliminators—Install high-efficiency drift eliminators on aerosol-generating water-cooled equipment to reduce release of bacteria in exhaust air streams.

10.2.1.3 Location of Equipment Exhausts—Locate exhausts from water-cooled heat-transfer systems so as to avoid entrainment of contaminants into the outdoor air intakes of HVAC systems serving the same and neighboring buildings (see 10.5.1).

10.2.2 Equipment Operation, Maintenance, and Inspection—Consult equipment suppliers or manufacturers to learn how to operate water-cooled heat-transfer equipment properly and follow these instructions. Appropriate use of scale and corrosion inhibitors, anti-foaming agents, and biocides (see 10.2.3) may be part of routine operation and maintenance. Good maintenance of water-cooled heat-transfer systems may not only limit legionella multiplication but also may improve equipment efficiency and extend service life. Inspect water-cooled heat-transfer systems regularly (for example, weekly or monthly) to confirm that equipment is operated and maintained as intended.

10.2.3 Biocide Use—The addition of chemical biocides

often is necessary to control multiplication of bacteria, protozoa, and algae in water-cooled heat-transfer systems, however, control of other microorganisms does not necessarily indicate control of legionellae. Obtain information on appropriate biocide selection and use for legionella control from equipment manufacturers or from companies experienced with the particular system in question. Consider the simultaneous use of more than one biocide and automatic biocide delivery. Refer also to Test Method E 645 and Guide E 1427.

10.2.4 Periodic Cleaning—Clean and disinfect water-cooled heat-transfer equipment periodically, for example, monthly, quarterly, bi-annually, or annually, as needed. Cleaning may entail physical or chemical removal of sludge, sediment, biofilm, algae, fungi, rust, scale, or corrosion. Clean and disinfect new equipment and equipment idle for extended periods, for example, six or more months. Drain and clean systems before shut down.

10.2.5 Recordkeeping—Develop detailed descriptions of all water-cooled heat-transfer systems, including all equipment cooled by a system and details of the make-up water supply. Keep, readily available, written procedures describing proper system operation and maintenance and indicating use of scale and corrosion inhibitors, anti-foaming agents, and biocides (see 10.2.2 and 10.2.3). Record the dates and results of equipment inspections, maintenance, cleaning, and testing (see 10.2.2, 10.2.4).

10.3 Control Measures for Potable Hot Water Supplies:

10.3.1 Hot Water Tank Capacity—See that the capacity of hot water tanks meets a facility's needs. Excessive hot water demand can result in delivery of insufficiently heated water, whereas demand far below available capacity can result in water stagnation. Design systems to circulate hot water, if possible, and minimize dead legs to reduce water stagnation.

10.3.2 Hot Water Temperature—Maintain hot water storage temperature at or above 60°C and deliver water to all outlets at or above 50°C, if permitted. Employ appropriate safeguards, for example, thermostatically controlled mixing valves, if scalding is a concern as a result of delivering water above 50°C.

10.3.3 Insulating Water Lines—Physically separate hot water lines running near cold water lines or insulate hot water lines to reduce heat transfer. Insulating hot water lines also helps maintain distribution and delivery temperatures (see 7.3.1.3).

10.3.4 Gaskets, Sealants, and Plumbing Fixtures—Do not use materials known to encourage biofilm formation or legionella multiplication (for example, natural rubber and silicone) in gaskets, seals, or plumbing fixtures for potable hot water supplies.

10.3.5 Unused Equipment—Flush unused hot water tanks, delivery lines, and similar equipment periodically (for example, monthly, bi-annually, or annually) or disconnect them from the main water supply and drain them (see 7.3.1.1).

10.3.6 Heat Shock Pasteurization—It may be advisable, in facilities such as health-care settings, periodically (for example, monthly, bi-annually, or annually) to pasteurize hot water systems by heating the water to at least 70°C for 2 to 24 h and flushing each outlet for at least 5 min with the

superheated water (see also 10.3.7).

10.3.7 *Chlorination*—It may be advisable, in facilities such as health-care settings, periodically (for example, monthly, bi-annually, or annually) to shock-chlorinate hot water systems by raising the free residual chlorine concentration to 10 mg L⁻¹ (ppm) and flushing each outlet until the odor of chlorine is detected (see 10.3.6). Chlorine is corrosive and will shorten the service life of metal plumbing. Chlorine's biocidal activity is sensitive to pH, decreasing rapidly above pH 7. Therefore, adjust pH to between 6 and 7 to use the lowest effective dose of chlorine. Refer also to Test Methods D 512, D 1067, and D 1293.

10.4 *Control Measures for Potable Cold Water Supplies:*

10.4.1 *Cold Water Tank Capacity*—See that the capacity of cold water tanks meets a facility's needs and that storage time does not exceed 24 h. Demand far below available cold water capacity when a tank is not well insulated can result in water stagnation and elevated temperatures (see 10.4.2).

10.4.2 *Cold Water Temperature*—Keep cold water temperatures at or below 20°C to limit legionella multiplication.

10.4.3 *Insulating Water Lines*—Physically separate cold water lines running near hot water lines, or insulate hot water lines to reduce heat transfer (see 7.3.1.3).

10.4.4 *Gaskets, Sealants, and Plumbing Fixtures*—Do not use materials known to encourage biofilm formation or legionella multiplication (for example, natural rubber and silicone) in gaskets, seals, or plumbing fixtures for potable cold water supplies.

10.4.5 *Cross Contamination*—Avoid contamination of cold water supplies by contact with water from other systems. Protect all connections to non-culinary processes using approved plumbing devices, for example, back-flow preventers or air gaps (see 7.3.1.2).

10.4.6 *Unused Equipment*—Flush unused cold water tanks, delivery lines, and similar equipment periodically (for example, monthly, bi-annually, or annually), or disconnect them from the main water supply and drain them (see 7.3.1.1).

10.4.7 *Chlorination*—It may be advisable, in facilities such as health-care settings, periodically (for example, monthly, bi-annually, or annually) to shock-chlorinate cold water systems by raising the free residual chlorine concentration to 20 mg L⁻¹ (ppm) for 2 h or to 50 mg L⁻¹ for 1 h. Run all outlets until the odor of chlorine is detected and leave the hyperchlorinated water in the system for the time stated above before flushing with fresh water. Chlorine is corrosive and will shorten the service life of metal plumbing. Chlorine's biocidal activity is sensitive to pH, decreasing rapidly above pH 7. Therefore, maintain pH between 6 and 7 to use the lowest effective dose of chlorine. Refer also to Test Methods D 512, D 1067, and D 1293.

10.5 *Control Measures for Heating, Ventilating, and Air-Conditioning (HVAC) Systems:*

10.5.1 *Location of Outdoor Air Intakes*—Locate outdoor air intakes for HVAC systems at a sufficient height and distance from possible sources of airborne bacteria (for example, exhausts from water-cooled heat-transfer equipment) and from possible sources of dust and debris to minimize entrainment of contaminants (see 7.3.4, 7.3.5.2, 10.2.1.3).

10.5.2 *Humidifiers*—Use humidifiers that emit water vapor or steam rather than ones that produce water droplets, that is, mists, if possible. Follow manufacturers' directions on cleaning and disinfecting humidifiers.

10.5.3 *HVAC Reservoirs and Condensate Trays*—HVAC equipment that allows water to collect may provide a location for microbiological growth, but the water temperature in these sources typically does not encourage legionella multiplication (see 6.1.4). Water in HVAC system reservoirs and condensate trays likely does not present a hazard of legionella transmission unless there is a mechanism for bacterial aerosolization from the source (see 6.3.4 and 6.3.5).

10.5.4 *Recordkeeping*—Develop detailed descriptions of all HVAC systems identifying the equipment in use and the parts of the facility each unit serves. Record the dates and results of equipment inspections, maintenance, cleaning, and testing.

10.6 *Control Measures for Spas, Whirlpool Baths, and Jacuzzis*—Typical water temperatures in these systems range between 32 and 40°C, which is in the temperature range that favors legionella multiplication (see 6.1.4). See that systems comply with applicable microbiological standards and recommended maintenance programs including biocide use and regular (for example, weekly or monthly) cleaning. Backflush, disinfect, or change water filters periodically (for example, weekly or monthly) to prevent excessive buildup of organic material. Control foaming to reduce bacterial release from bursting bubbles.

10.7 *Control Measures for Decorative Fountains*—Keep decorative fountains and similar equipment clean and operate and maintain such equipment according to the designer's or manufacturer's instructions including chlorination or other water treatment.

10.8 *Protocol for Managing a Legionella-Related Emergency*—Refer also to the CDC Guideline for Prevention of Nosocomial Pneumonia and (3) for information on managing a legionella-related emergency.

10.8.1 Develop protocols for dealing with emergencies such as the need to shut down equipment or take water systems out of service quickly. Such emergencies may arise when water systems are implicated in legionella transmission or are found to support undesirable concentrations of the bacteria (see 6.2.4.2). Include in emergency protocols information on where to obtain building plans, directions on arranging meetings with facility staff and occupants and the local community, identification of persons in charge of coordinating media contacts (for example, television, radio, and newspapers), and a mechanism to obtain and to disseminate accurate information to answer basic questions and to address concerns.

10.8.2 Include in emergency plans a list of who to contact at the local health department and information on where to obtain replacement equipment and supplies to clean and disinfect water systems. Coordinate with public health authorities emergency activities such as the shutting down, testing, disinfecting, draining, and cleaning of equipment implicated in legionella transmission. Collect water samples for legionella detection (see Section 8) before treating implicated equipment to provide baseline information on water quality (see Section

10) and to obtain environmental legionella isolates for comparison with clinical isolates (see 6.2.1, 6.6.2.1). Also collect water samples after completion of control measures to determine their success in reducing or eliminating legionellae (see 10.1-10.7, and Section 11).

11. Follow-up of Environmental Inspections and Epidemiological Investigations

11.1 Repeat or continue cleaning and disinfection measures on water systems (see Section 10) until the desired environmental control of legionellae is achieved (see 5, 6.2.4, 7). Continue epidemiological surveillance for legionellosis (see

9.3 and 9.4) for up to 12 months after implementation of control measures to identify new infections as soon as possible should they occur.

12. Keywords

12.1 epidemiological investigation; heating; ventilating; and air-conditioning systems; Legionnaires' disease; legionella; *Legionella pneumophila*; legionellosis; legionellosis surveillance; outbreak investigation; microbiological water monitoring; Pontiac fever; water-cooled heat-transfer equipment; water sampling; water supplies; water systems; water system inspection

APPENDIX

(Nonmandatory Information)

X1. SAMPLE OF LEGIONNAIRES' DISEASE QUESTIONNAIRES

X1.1 Figs. X1.1 and X1.2 provide sample Legionnaires' disease questionnaires.

Interviewer's name: _____ Interview date: ____/____/____

We at (identify the public health office) are investigating a cluster of respiratory infections at a local (workplace/school/etc.). (Workplace/school/etc.) records show that you may have been absent two or more days in a row in the past two months. If now is a convenient time, I would like to ask a few questions about your absences.

1. Name (last): _____ (first): _____
 2. Date of birth: _____ 3. Gender: M F
 4. Home phone: _____ 5. Work phone: _____

6. Have you been absent two or more days in a row in the past two months?: Yes No

If No, conclude the interview; if Yes, continue with the following questions.

7. Date(s) of absence: _____
 8. Reason(s) for absence: Illness _____ Injury _____ Pregnancy _____
 Surgery _____ Other _____

If absence not due to Illness, conclude the interview; if absence due to Illness, continue.

9. When did you first become ill?: ____/____/____
 10. Have you recovered?: Yes No If Yes, how many days were you ill?: _____ days
 11. Did you experience any of the following symptoms during your illness?:
 Fever ($\geq 38^{\circ}\text{C}$): Yes No Unsure If Yes, highest temperature: _____ $^{\circ}\text{C}$
 Cough: Yes No Unsure If Yes, was the cough productive?: Yes No
 12. Did you see a doctor for this illness?: Yes No

If No, conclude the interview; if Yes, continue.

13. On what date(s) did you see a doctor?: _____
 14. What did the doctor tell you about your condition?: _____

15. Were you admitted to a hospital?: Yes No
 If Yes, what hospital and when: _____

16. If necessary, may we contact your doctor regarding this illness?: Yes No
 17. Doctor's name and phone number: _____

FIG. X1.1 Sample—Legionnaires' Disease Surveillance Questionnaire

Interviewer's name: _____ Interview date: ____/____/____

We at (identify the public health office) are investigating a cluster of respiratory infections at a local (workplace/school/etc.) (Patient's name) reports that (he/she) saw you on (date) because of an illness. I would like to ask a few questions about your evaluation of this patient.

Patient's name: _____ Date of birth: ____/____/____

1. Did the patient have pneumonia?: Yes No

If No, conclude the interview; if Yes, continue with the following questions.

2. What etiology did you identify for the pneumonia?: _____

3. Was a chest x-ray taken? Yes No Not known

If Yes, what were the findings?: _____

4. Was a specimen collected for culture?: Yes No Not known

If Yes, when was it collected, where was it tested, and what were the results?:

Date: ____/____/____ Laboratory: _____

Results: _____

5. Was a specimen collected specifically for legionella culture?: Yes No Not known

If Yes, when was it collected, where was it tested, and what were the results?:

Date: ____/____/____ Laboratory: _____

Results: _____

6. Was a specimen collected for legionella DFA testing?: Yes No Not known

If Yes, when was it collected, where was it tested, and what were the results?:

Date: ____/____/____ Laboratory: _____

Results: _____

7. Was a blood sample collected for legionella serological testing?: Yes No Not known

If Yes, when was it collected, where was it tested, and what were the results?:

Date: ____/____/____ Laboratory: _____

Results: _____

8. Was a urine sample collected for legionella antigen testing?: Yes No Not known

If Yes, when was it collected, where was it tested, and what were the results?:

Date: ____/____/____ Laboratory: _____

Results: _____

FIG. X1.2 Sample—Legionnaires' Disease Health Care Provider Questionnaire

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