ACUTE COMMUNICABLE DISEASE CONTROL PROGRAM

Guidelines on the Surveillance, Investigation, and Control of Legionellosis in Los Angeles County

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COUNTY OF LOS ANGELES Public Health

LOS ANGELES COUNTY HEALTH AGENCY

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Table of Contents

Table of Contents (cont.)

Guidelines on the Surveillance, Investigation, and Control of Legionellosis in Los Angeles County

I. Purpose.

The Los Angeles County (LAC) Department of Public Health (DPH) *Guidelines on the Surveillance, Investigation, & Control of Legionellosis in Los Angeles County* (hereinafter Guidelines) provide direction to LAC DPH staff using evidenced-based standards of practice regarding the public health activities to reduce risk of Legionnaires' disease in Los Angeles County.

II. Introduction.

A. Background: LAC DPH Acute Communicable Disease Control program (ACDC) routinely conducts *Legionella* investigations in collaboration with our partner LAC public health programs, Environmental Health (EH) program and the Public Health Laboratory (PHL). We want to ensure that the process of conducting these investigations, and the role of each entity, is clearly defined and standardized. Staff from ACDC, EH, and the PHL convened a work group to understand each program's roles and responsibilities inherent in these investigations. The work group's goals were to develop standards and guidance to manage an environmental investigation of a healthcare facility that is associated with a confirmed and/or suspected case(s) of legionellosis, to define program roles, and most importantly, to ensure the absence of conditions that foster *Legionella* bacteria amplification that may lead to disease transmission.

B. Goals: Legionellosis is a reportable disease in LAC per Title 17 California Code of Regulations (CCR) § 2500 [1] and 2505 [2]. Healthcare providers, including laboratories, are mandated to report a case, suspected case, and/or outbreak associated with this organism. As new knowledge becomes available through our local experience, collaboration with state and federal agencies, and through the literature, it is our hope that these guidelines will provide direction to LAC DPH staff to ensure the safety LAC residents and visitors.

III. Clinical Manifestations.

Legionellosis is a severe, potentially fatal infectious disease that is caused by many pathogenic *Legionella* species (spp.) and is associated with two clinically and epidemiologically distinct illnesses: Legionnaires' disease and Pontiac fever. Legionnaires' disease is the more severe form of legionellosis and is characterized by fever, myalgia, cough, and clinical or radiographic pneumonia 2–10 days after exposure. Legionnaires' disease causes death in up to 5–30% of cases [3]; although, most cases can be successfully treated with antibiotics. Pontiac fever produces a milder flu-like, non-pneumonic illness, occurring within a few hours to 2 days of exposure. It is a self-limited illness that requires no treatment and most commonly occurs in persons who are otherwise healthy.

IV. Etiology.

Legionella spp. are Gram-negative, aerobic, non-spore forming bacteria with at least 60 species and multiple serogroups identified. *Legionella pneumophila* serogroup 1 (*Lp*1) is responsible for a greater frequency of infections. *Legionella* spp. are naturally found in warm, aquatic environments, replicate in ciliated protozoa and amoebae in the environment and in alveolar macrophages in humans [4], thrive in warm temperatures 25°C–42°C (77°F–108°F), and are relatively resistant to the effects of chlorine and heat [5].

V. Epidemiology.

A. Since *Legionella* bacteria are widespread in natural and human-made environments, they can enter facilities in small numbers through the water system and can then proliferate within the biofilm of the water supply system in areas of the facility. Such areas include but are not limited to cooling towers, domestic hot water systems, spas, air conditioning systems, swimming pools, fountains, and showers, most of which have been sources of legionellosis outbreaks. The importance of robust surveillance for healthcare associated infections (HAI) of legionellosis, even in the setting of an environmental control and prevention program, should be highlighted. Clinical surveillance with careful attention to healthcare exposures during the incubation period should remain an important component [6].

B. *Legionella* transmission occurs through the inhalation of aerosolized droplets containing the bacteria and aspiration of contaminated water. Person-to-person transmission does not occur. Sites typically implicated in outbreaks are group residences, meeting rooms, dormitories, hotels, hospitals, cruise ships, gyms, spas, and other facilities where there is a gathering of people and large, complex water systems [4].

C. Detection of *Legionella* spp. from an environmental source does not always indicate an increased risk of infection or transmission of disease. Risk of illness is associated with many factors such as strain virulence, susceptible populations, and effective aerosolization and aspiration. The disease occurs most commonly in older adults (50 years of age or older) and among immunocompromised people. Smokers and persons with chronic diseases such as diabetes, renal or hepatic impairment have higher risk, as well as persons with chronic lung disease, such as emphysema and chronic obstructive pulmonary disease. Infection in children is rare and usually is asymptomatic, or mild and unrecognized. Severe disease has occurred in immunocompromised children and those with end stage renal disease or underlying pulmonary disease. Recent travel involving overnight stays and stays in healthcare facilities, and exposure to hot tubs are other associated risk factors [4].

VI. Case Evaluation and Definitions.

A. Case Evaluation: The ACDC Liaison Public Health Nurse (LPHN) on the ACDC Healthcare Outreach Unit (HOU) reviews the patient's clinical, radiographic, and microbiologic information and classifies reported cases of legionellosis using the definitions of suspected versus confirmed, healthcare-associated, and/or travel-associated cases provided by the Centers for Disease Control and Prevention (CDC) and the California Department of Public Health (CDPH) [7, 8, 9]. The CDC and CDPH case definitions for Legionnaires' Disease (LD) includes a compatible clinical history (see clinical manifestations) and meets at least one of the following criteria.

B. Definitions

1. **Suspected** [7, 9]
   a. By seroconversion: fourfold or greater rise in antibody titer to specific species or serogroups of *Legionella* other than *L. pneumophila* serogroup 1 (e.g., *L. micdadei, L. pneumophila* serogroup 6, etc.).
   b. By seroconversion: fourfold or greater rise in antibody titer to multiple species of *Legionella* using pooled antigen and validated reagents.
   c. By the detection of specific *Legionella* antigen or staining of the organism in respiratory secretions, lung tissue, or pleural fluid by direct fluorescent antibody (DFA) staining, Immunohistochemistry (IHC), or other similar methods, using validated reagents.
   d. By detection of *Legionella* species by a validated nucleic acid assay.
2. **Confirmed** [7, 9]
   a. By culture: isolation of any *Legionella* organism from respiratory secretions, lung tissue, pleural fluid, or other normally sterile fluid.
   b. By detection of *Legionella pneumophila* serogroup 1 antigen in urine using validated reagents.
   c. By seroconversion: a fourfold or greater rise in specific serum antibody titer to *Legionella pneumophila* serogroup 1 using validated reagents.
3. **Travel-associated** [8]
   a. A confirmed LD case that has a history of spending at least one night away from home, either in the same country of residence or abroad in the 10 days before onset of illness. If the case spent the night at a residence, the mailing code where the case spent the night should also be obtained.
   b. If a case is travel-associated, a detailed case summary (see appendices) is prepared by the ACDC Healthcare Outreach Unit (HOU) liaison public health nurse (LPHN) and emailed to the California Department of Public Health (CDPH) within 7 days of the initial notification. The state health department will then report the case to the CDC. If the CDC identifies any epidemiologically linked travel-associated legionellosis cases, a notification about the case will be sent to the public health department of the state(s) where the case visited to help identify possible outbreaks.
4. **Healthcare-associated** [8, 9]**:** HOU LPHN will assess healthcare-associated infection (HAI) transmission utilizing the following CDC case definitions:
   a. Definite HAI LD case: Laboratory confirmed diagnosis and case residing in a healthcare facility continuously for ≥ 10 day before the onset of symptoms. LAC DPH further classifies HAI cases into the following two categories:
      i. High-probability definite HAI: Patient was hospitalized or a resident in *only one* healthcare facility during the entire 10-day incubation period. Each overnight stay is in the same facility. Therefore, the definite HAI case has a higher probability of having been exposed to *Legionella* at the facility in question.
      ii. Low-probability definite HAI: Patient was hospitalized or a resident in *more than one* healthcare facility during the entire 10-day incubation period. Each overnight stay was in a healthcare facility, but not necessarily the same healthcare facility. The definite HAI case has a lower probability of having been exposed to *Legionella* at any one of the multiple facilities in question; therefore, LHD cannot say with high probability that the patient was exposed to *Legionella* in one over another facility.

b. Possible HAI LD case: Laboratory confirmed diagnosis and case residing in a healthcare facility for one or more nights during the 2–9 days before the onset of symptoms.
c. Per Centers for Medicare and Medicaid Services (CMS), hospitals, critical-access hospitals, and skilled nursing facilities are required to have a water-management program in order to reduce the risk of waterborne pathogens, which includes reducing the risk of *Legionella* [10].

5. **Community-acquired:** Diagnosis is made within the first 2 days of hospitalization, and the case did not reside in a healthcare facility at any time in the 10 days prior to the onset of symptoms.
6. **Outbreak**:
   a. Per CDC, an outbreak is defined as two or more confirmed LD cases where the onset of illness is closely linked in time and in space, where there is suspicion of, or evidence of, a common source of infection, with or without microbiological support [11].
   b. For the purposes of investigation, the terms “outbreak” and “cluster” are used interchangeably.
   c. For the purposes of investigation, the terms “full investigation” and “outbreak investigation” are used interchangeably.
   d. Healthcare-associated outbreak investigations:
      i. One high-probability definite HAI LD case may warrant a full investigation.
      ii. Two or more low-probability definite HAI LD cases, or two or more possible HAI LD cases, may warrant a full investigation. Per CDC statement on considerations for the LHD [12], the decision to conduct a full investigation is made on a case-by-case basis by ACDC.
   e. Community-associated outbreak investigations: DPH will evaluate and determine community-associated outbreak investigations on a case-by-case basis [12].

VII. Investigation of Confirmed Legionnaires’ Disease Cases.

A. ACDC, Healthcare Outreach Unit, Liaison Public Health Nurse Role: DPH receives reports of suspect legionellosis cases via the electronic laboratory reporting system (ELR), web-based confidential morbidity reporting system (CMR), telephone calls and reports from health professionals, other health jurisdictions, and the public. The ACDC HOU LPHN obtains medical records for reported legionellosis cases, interview case(s) utilizing the CDPH Legionellosis Case Report form [13]. If the case is unable to be interviewed, the HOU LPHN will try to interview a significant other, family member, or medical decision-maker who is knowledgeable about the case's history, as appropriate. The HOU LPHN submits the form to the ACDC Morbidity team once the interview is completed, and the case-report form is then forwarded to CDPH. The HOU LPHN records demographic and epidemiologic data into vCMR and into the HOU *Legionella* database.

B. Special Considerations: LPHN assess for healthcare-associated infections (HAI), travel-associated cases, and potential community-associated outbreaks. The HOU LPHN will notify the ACDC HOU Supervising Physician of any suspected HAI case or community outbreak. For travel-associated cases, HOU LPHN notifies CDPH staff, via secure encrypted email. The decision to pursue a full investigation is made on a case-by-case basis, taking into consideration many factors and possible sources of exposures.

C. Determination of DPH Investigation into HAI LD Case(s): A complete assessment to determine the likelihood that a confirmed HAI Legionnaires' case was acquired at the facility should be performed prior to initiating a full investigation. As described above, a healthcare-associated case may be classified *high-probability* definite or *low-probability* definite HAI LD case. The following factors may be considered in ACDC's assessment process:

1. Potential *Legionella* exposures outside the facility: Patients with Legionnaires' disease often will have traveled outside the facility during the potential incubation period, leading to other potential *Legionella* exposures. A full history of the number and nature of these exposures outside of the facility should be obtained, particularly with any travel that may have involved exposure to a water source. If additional potential exposures outside the facility are identified, DPH may elect to monitor for additional cases at the facility in question.
2. Patients with Legionnaires' disease frequently have a chronic medical history, including underlying pulmonary disease and are at risk for either repeated pulmonary infections or exacerbations of underlying illness. The patient's medical records from other facilities should be obtained and reviewed, as other events may mimic Legionnaires' disease. *Legionella* urine antigen may remain positive for months after illness; therefore, patients could have multiple episodes of illness in the period prior to *Legionella* testing, making it difficult to establish the actual illness onset date.
3. Time delay until case identification: A time lag of up to 2 months will frequently occur from a patient's symptom onset until confirmation of healthcare-associated Legionnaires' disease. If the facility has conducted adequate surveillance in that time frame and no additional cases have been identified, the LHD may decide not to conduct a full investigation.
4. Clinical character of a resident population: Facilities with many high-risk patients, such as elderly or immunocompromised, may be judged at higher risk and may merit a full investigation at a lower threshold.
5. Facility surveillance capacity: If the facility has an effective surveillance plan such that suspect Legionnaires' disease cases are likely to be identified quickly, DPH may consider simply continuing surveillance.
6. Previous testing of the site: If previous testing has indicated either positive results for *Legionella* or change in water quality (e.g. low chlorine levels), this may indicate the need for a full investigation.
7. If the assessment does not indicate with certainty that the facility was the source of infection, ACDC may elect to continue enhanced surveillance without initiating a full investigation.
8. Enhanced surveillance by the HOU LPHN may include but is not limited to the following:
    a. Request that the health facility's Infection Preventionist (IP) conduct a 6-month retrospective review of patients throughout the facility with healthcare-associated pneumonia (pneumonia that develops day 3 or later after admission), and continue enhanced surveillance for healthcare-associated pneumonia for 6 months prospectively.
    b. A retrospective review will include all cases diagnosed with healthcare-associated pneumonia six (6) months prior to the date of symptom onset of the current possible or definite HAI Legionnaires' disease case.

c. A prospective review will include all cases diagnosed with healthcare-associated pneumonia six months (6) prospectively from the date of symptom onset of the current possible or definite HAI Legionnaires' disease case.
d. Instruct the IP that *Legionella* urine antigen tests and respiratory cultures tested specifically for *Legionella* on appropriate media should be obtained for all cases with healthcare-associated pneumonia. Per CDC, *Legionella* obtained from respiratory sputum may be isolated on Buffered Charcoal Yeast Extract (BCYE) agar [14].
e. Instruct the IP to notify ACDC immediately of any additional suspect legionellosis cases found on retrospective and prospective review of cases. If additional cases are facility linked, a full investigation will be conducted.

9. Consider a full investigation for a single case of possible HAI Legionnaires' disease depending on the level of patient risk, such as patients with a diagnosis of malignancy and those on oncology wards. A full investigation should always be performed following a single case of possible HAI Legionnaires' disease on a hematopoietic stem cell (bone marrow) or other organ transplant unit. In a group-residential or community facility exposure, the HOU LPHN will monitor these facilities for a year, and if additional confirmed cases are later linked to the facility in question, then a full investigation will be conducted at that time.

D. Determination of DPH Investigation into Community Outbreak: DPH will evaluate suspect community outbreaks on a case-by-case basis to determine if a full investigation is warranted.

VIII. Full Investigations.

A. DPH may decide to conduct a full investigation as a best practice to reduce the risk of ongoing *Legionella* transmission to other healthcare-facility patients and residents, as well as any staff and visitors to these facilities.
1. A patient may have a complex medical history with recent respiratory illness and may have been exposed elsewhere. A *Legionella* urinary antigen test may remain positive for prolonged periods, thereby making it difficult to conclude with confidence that a positive result is associated with exposure at a particular facility.
2. Perform a full investigation at a facility for the source of *Legionella* with:
   a. ≥ 1 case(s) of high-probability definite HAI LD;
   or
   b. ≥ 2 cases of low-probability HAI or possible HAI LD identified within 6 months of each other ***and*** DPH finds there is a high probability that exposure to *Legionella* occurred at the identified facility or facilities.
3. Epidemiological data of cases are considered by DPH prior to the on-site investigation and environmental assessment. These data and other, supplemental information that may have been received from the facility will guide the full investigation and the locations from which the samples are collected. The assessment should focus on possible water sources and aerosolized water.

B. Full Investigation Protocol:
1. Formation of the **DPH Investigation Team**: The DPH Investigation Team, comprising subject matter experts from ACDC, Environmental Health (EH) and other stakeholders, as needed, will perform the on-site investigation.

Representatives from several EH teams will be included, as appropriate, depending on the type of facility and hypothesized exposures. These may include the Bureau of Environmental Protection Drinking Water Program (water quality), Cross Connections and Water Pollution Control Program (plumbing systems), Recreational Waters Program (pools and spas), Bureau of Toxicology and Environment Assessment (air handling), Housing Institutions (jails and housing facilities). Depending on the situation, Los Angeles County Public Health Laboratory (PHL), the local municipality water district, and the DPH Emergency Preparedness and Response program may each provide additional support, which potentially includes on-site participation in the investigation.

2. ACDC also informs the Los Angeles County Health Facilities Inspection Division (HFID), which collaborates closely with CDPH Licensing and Certification, of the Legionnaires' disease cluster, suspect LD outbreak, and the resulting investigation. A joint site investigation with HFID will be requested. HFID and/or CDPH Licensing and Certification may opt to investigate independently of ACDC, as they deem necessary.
3. ACDC will convene the DPH Investigation Team and will lead the initial full investigation. ACDC will coordinate with the members of the DPH Investigation Team via phone and email to share information, formulate a hypothesis, and develop an action plan for the investigation. HFID will be included for acute care hospitals.
4. The DPH Investigation Team will determine suspected exposure and water source locations where water samples are to be collected; supplemental samples may be recommended by EH.
5. Designated EH program staff who are assigned to collect water samples during the on-site investigation will first retrieve the appropriate sampling materials from PHL (if PHL does not participate in the site visit).
6. The DPH Investigation Team will conduct the on-site investigation, which will be scheduled within five business days of the DPH Investigation Team initial call.

C. Full Investigation General Action Plan (refer to **Appendix A: ACDC *Legionella* Action Plan and Checklist for Full Investigations**)

1. ACDC will be the initial point of contact with the facility.
2. ACDC will lead the entrance and exit conferences with the facility.
3. Designated EH program staff will collect water samples and appropriate facility reports (e.g. site plan, plumbing and heating, air conditioning, and ventilation [HVAC] schematics, etc.).
4. ACDC may conduct a review of medical records related to suspect cases (if investigating a healthcare facility).
5. ACDC will issue initial findings and interim recommendations to the facility at the exit conference. If EH or HFID, or both, are involved in the on-site investigation, program staff may also issue their initial findings and interim recommendations at the exit conference.
6. ACDC recommendations may include the following:
   a. Discontinue using potential water source(s) of *Legionella* until ACDC/EH notification (pending water-testing results).
   b. Perform corrective measures as recommended aimed at maintaining undetectable levels of *Legionella* spp., which is the ACDC goal during outbreak investigations.

c. Post a *Legionella* notification letter for patients/residents, visitors, and staff in a conspicuous location at the facility being investigated (e.g. spa or hospital entrance; see appendices).
d. Comply with additional recommendations outlined by the DPH Investigation Team.
e. Recommend that the facility consider hiring a consultant with expertise in reducing *Legionella* risk in water-management systems in that type of facility.
f. Submit the facility's water-management program (WMP) to ACDC for review

7. ACDC will email summary of initial findings of the DPH Investigation Team interim recommendations discussed at the exit conference to the facility contact within 1–2 business days.

IX. Environmental Health: Facility and Environmental Assessment

A. Laws and Regulations: In addition to ACDC, Environmental Health shall have the authority vested in the Los Angeles County Health Officer to conduct *Legionella* investigations. In some cases, the following Health and Safety codes are applicable: California Health and Safety Code, California Code of Regulations, Title 22 and 24, Los Angeles County Code Title 11, and the Uniform Plumbing Code, Title 17, California Code of Regulations, Section 2501.

B. Refer to the following standards, guidelines, and resources:
1. *ANSI/ASHRAE Standard 188-2018 -- Legionellosis: Risk Management for Building Water Systems* [15];
2. *ASHRAE Guideline 12-2000 -- Minimizing the Risk of Legionellosis Associated with Building Water Systems* [16];
3. CDC, National Center for Immunization and Respiratory Diseases, Division of Bacterial Diseases, *Sampling procedure and potential sampling sites: Protocol for collecting environmental samples for* Legionella *culture during a cluster or outbreak investigation or when cases of disease may be associated with a facility* [17];
4. CDC, Environmental Assessment of Water Systems [18];
5. CDC, *Guidelines for Environmental Infection Control in Health-Care Facilities: Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC)* [19];
6. CDC, *Legionella* (Legionnaires' Disease and Pontiac Fever): Environmental *Legionella* isolation techniques evaluation program [20];
7. United States Department of Labor Occupational Safety & Health Administration, OSHA technical manual section III, chapter 7: Legionnaires' Disease [21].

C. Information Gathering:
1. Prior to the facility on-site assessment, the following information is vital to environmental health staff, and shall be gathered:
2. Inquire about any interim remedial measures that may have been taken by the facility that could impact the environmental sampling plan
3. Identify possible amplification sites that also pose the risk of aerosolizing water droplets, which may include:
a. Stagnant water and/or plumbing designs and installations that result in stagnation (side-arm and dead leg piping);
b. Warm water temperatures between 20°–50°C (68°–122°F);
c. Bulk water pH from 5.0–8.5;
d. Sediment, scale, deposits, and biofilm, which support not only *Legionella* growth, but also the growth of the supporting microbiota for *Legionella*

(including algae and many bacteria that supply essential nutrients for growth). Certain amoebae and other protozoa harbor *Legionella* as endosymbionts, allowing them to thrive, resist harsh environmental conditions (including biocides), and to amplify.

4. Identify water systems that can serve as *Legionella* amplifiers and disseminators and have been associated with Legionnaires' disease (potable water distribution systems, hot water heaters, and cooling towers). They include:
   a. Domestic hot water systems (tap faucets, showerheads, sprayers) with water heaters that operate below 60°C (140°F) and deliver to taps below 50°C (122°F). EH should consider the following:
      i. Supplier of the incoming water main,
      ii. Monochloramine,
      iii. Free or combined chlorine residual,
      iv. The temperature at proximal and distal locations,
      v. Re-circulation,
      vi. Backflow prevention,
      vii. Water heaters or break tanks,
      viii. Dead legs in plumbing design,
      ix. Shower head design and location.
   b. Cooling towers and evaporative condensers
      i. *Legionella* may be carried in cooling tower aerosols for distances of up to 2 miles. EH to assess the presence of cooling towers within surrounding land use;
      ii. Visible condition to include evidence of algae growth, biofilm, rust, scale and other signs of contamination or poor maintenance;
      iii. Are control measures used
         - Name of product
         - Active ingredient
         - Concentration (determined by field test);
      iv. Water temperature;
      v. Dates tower was operated;
      vi. Date tower was last serviced, frequency of service, and results
      vii. Service company information to include laboratory results for total bacteria in water reservoir (if available);
   c. Spas and whirlpools
      i. Visible condition
      ii. Chlorine/bromine concentration, pH, cyanuric acid levels
      iii. Types of filters
      iv. Date last backwashed
      v. Date last drained and scrubbed
      vi. Temperature
      vii. Visible biofilm layer (check skimmer basket)
      viii. Fill water supply
      ix. Maintenance records
      x. Ventilation efficiency: verify exhaust flow rate and dedicated 100% outdoor exhaust;
   d. Humidifiers and decorative fountains
      i. Visible condition include proximity to the outdoor air intake
      ii. Are control measures used
         - Name of product

- Active ingredient
- Concentration (determined by field test)

iii. Water supply
iv. Filters;

e. Water fountains;
f. Ice machines;
g. Reservoir misters (designated EH program to inspect);
h. Respiratory and dental therapy equipment (designated EH program to inspect);
i. Ultrasonic mist machines;
j. Damp potting soil;
k. Other sources: fire sprinkler systems, eyewashes/safety showers, dental water line
   i. Date last flushed
   ii. Temperature.

5. Request and review plumbing diagrams of the building
6. Request and review the water management program (WMP) including water treatment reports if available (may include cooling tower, pool/spa, hot water, water mister, and fountain maintenance logs).
7. Identify the locations of outside air intakes for HVAC systems in relation to cooling towers or fountains and other water features capable of producing aerosols.
8. Inquire about, or review "as-built" mechanical plans for the presence of humidifiers within the HVAC systems loop, filtration efficiencies (e.g. air filters MERV rating).

D. Facility Planning:
1. Require the facility to provide reasonable access to the affected room(s), and any associated areas impacting the affected room(s) and water systems.
2. EH will lead the collection of environmental samples:
   a. Use the information gathered and PHL capacity to estimate how many samples to collect and of what type, in consultation with ACDC (e.g. swabs, liter bottles, etc.);
   b. Walk the affected facility to identify potential sources of exposure to *Legionella*;
   c. Determine the necessary types of equipment to conduct the facility assessment and safely gain access to the sampling sites. This may include: camera, flashlight, thermometers, humidity direct reading meter, smoke tubes, micro-manometer, pool/spa test kit, domestic water test kit for chlorine and pH, a quaternary ammonia test kit and tools to open inspection panels;
   d. Determine means to stop the aerosolization of water from potential amplification sites during the sample collection if identified;
   e. Identify means to prevent unexpected or unauthorized reactivation of pumps, fans, or other mechanical devices that aerosolize water (lock-out/tag-out);
   f. Determine if there have been any changes in the routine operation of the facility relevant to the incubation period of the case(s) (e.g. onsite construction, interruption in the water supply to the facility, under boil water notice, change in municipal water supplier, etc.).

E. General Instructions for Environmental Assessment:
1. Use CDC and other standardized forms to completely document the findings of the environmental assessment at the facility.
2. Interview any facility staff who may have knowledge of the water systems within the facility.

3. Inspect specific areas and document the conditions found within the facility that are the most likely source(s) of *Legionella.*
4. Conduct water sampling (designated EH program and trained staff—see below): See III Facility/Environmental Investigation Protocol, C. Environmental Health: Facility/Environmental Assessment, 4. Environmental Assessment Water Sampling. Also consider III Facility/Environmental Investigation Protocol, C. Environmental Health: Facility/Environmental Assessment, 1. Gathering Information.
5. Use appropriate personal protective equipment (PPE) when there is a significant risk of exposure to high concentrations of contaminated aerosols. *PPE is required when samples are collected since concentration and pathways of exposure are unknown.*

F. Environmental Assessment: Water Sampling.
1. EH staff (designated and trained) will select and complete water sampling within identified areas, as follows:
2. Room selection will be based on the location of case(s) occupancy/activity;
3. Obtaining water and other environmental samples will be based on the location of case(s) occupancy/activity, which may include the following:
   a. Potable water outlets including faucets and showerheads, as appropriate;
   b. Potable water systems including incoming water main, water softener, holding tanks, water heater tanks (at the inflows and outflows) as appropriate;
   c. Distal room on the riser based on the location of case(s) occupancy/activity, as appropriate;
   d. Cooling tower, air conditioning systems (evaporative condensers), including makeup water (added to replace water lost because of evaporation, drift, leakage), basin (area under the tower for collection of cooled water), sump (section of basin from which cooled water returns to heat source), heat sources (e.g. chillers), as appropriate;
   e. Humidifiers including bubblers for oxygen and water used for respiratory therapy equipment, as appropriate;
   f. Other sources as appropriate to include fountains, ice machines, irrigation equipment, fire sprinkler system (if recently used), and hot tubs [17].
4. A water sample will be taken from the return of the hot water loop of the riser where the case resided, if appropriate.
5. Water samples from locations in appropriate rooms may include:
   a. A sample of cold water will be taken on the first draw;
   b. A sample of cold water will be taken after a one-minute flush;
   c. A sample of hot water will be taken on the first draw;
   d. A sample of hot water will be taken after a flush lasting a few minutes;
   e. A sample of each fixture and the corresponding aerator will be performed.
6. Water sample collection data and log times will be documented.
7. Bulk water samples taken will be one liter (1L).
8. Water samples will be packed in insulated containers at ambient temperature and taken to the PHL by designated PH agency.

G. Results of Water Sampling:
1. If any *Legionella* spp. are identified, promptly institute water system decontamination measures [18]. This includes *Legionella* species other than *pneumophila.*

2. ACDC will continue surveillance for new cases and will recommend further remediation or decontamination pending identification of the source of *Legionella* spp.

H. Post-investigation Monitoring:
1. After results of environmental testing (e.g. PHL, third party) have been obtained and recommendations for remediation and other prevention strategies are provided to the facility, ACDC will close its investigation, but maintain contact with the facility during the prospective surveillance period. EH will verify corrective actions per the EH compliance letter is completed. Health Facilities will continue to monitor and work with the facility to ensure that recommendations for remediation were implemented appropriately.
2. If *Legionella* spp. are detected after remediation efforts, designated EH program/EH lead will reassess control measures, modify accordingly, and repeat the decontamination procedures; consider intensive use of techniques used in the initial decontamination or a combination of superheating and hyperchlorination [18].
3. If a third-party remediation company is hired by the facility, EH will work independently with the company until EH determines their role is no longer needed.
4. All violations\discoveries in relation to *Legionella* mitigation shall be forwarded to HFID according to CMS mandates.
5. EH will continue to provide ACDC with all laboratory re-culture test results, official communication/documents to the facility, and provide the final facility letter/report given to the facility. Subsequent cultures will be performed at a commercial lab using an ELITE certified commercial laboratory.

I. Facility Environmental Assessment Follow-up:
1. ACDC, EH, and PHL will discuss water sample results and determine a plan of action;
2. ACDC may contact other agencies (e.g. CDPH, CDC, HFID) for consultation;
3. ACDC will communicate the plan of action to the facility:
   a. ACDC, EH, and HFID will develop a comprehensive set of corrective orders and recommendations and will email and/or mail to the facility within 2 business days;
   b. ACDC, EH, and HFID will follow-up on their respective corrective orders and recommendations to ensure compliance.
4. ACDC will maintain results of all water samples, facility reports and communication while the investigation is open. EH will also maintain water sample results.
5. ACDC will provide an official final report (often in the form of a letter) to the facility when ACDC closes investigation (meaning thus that no additional cases were found upon the completion of the 6-month prospective review):
   a. Final report will be emailed and/or mailed to the facility within 3 months of the closure of the investigation;
   b. If further follow-up with the facility is required by EH or HFID after the ACDC investigation is closed:
      i. ACDC will inform EH and HFID that the investigation has been closed;
      ii. EH will provide their final report to the facility and to ACDC when the EH investigation is closed.
6. If HOU LPHN identifies any new cases on the prospective facility review of cases once ACDC and/or EH has closed their investigation, the LPHN will immediately inform the ACDC HOU Supervising Physician. As appropriate, ACDC may consult

with EH and HFID to determine a plan of action if additional actions may be warranted.

X. Short-term Remediation and Decontamination.

A. Facility Closure: If during or after the investigation, DPH or another regulatory agency determines that partial or complete closure of the facility is warranted, the program or agency that determined the need for closure will be responsible for determining criteria for re-opening the facility and identifying when those criteria have been met in consultation with ACDC, EH, PHL, HFID.

B. Short-term Mitigation Strategies: Options to minimize patient exposure to potable water sources:
1. Restrict patient showering until remediation strategies have been shown to be effective (strongly recommended).
2. Restrict drinking directly from potable water sources until remediation introduced; supply bottled water to patients.
3. Introduce point-of-use filters for faucets and showerheads.
4. Turn off and drain all decorative water features and whirlpool spas until remediation strategies have been shown to be effective. At that time, these items should be thoroughly disinfected and scrubbed before returning to service (strongly recommended).
5. Note: Some combination of these strategies should be implemented immediately and remain in place until a long-term remediation strategy has been shown to be effective.
6. Short-term systematic potable water system remediation: For detailed information see *ASHRAE Guideline 12-2000 -- Minimizing the Risk of Legionellosis Associated with Building Water Systems* [16].

C. Additional information is available in CDC's HICPAC guidelines beginning on page 10 [19].
1. Hyperchlorination to greater than or equal to 2 parts per million (ppm) at all distal sites and flushing at all points of use;

and/or

2. Superheating the potable water system to 160°F–170°F (hot water must be flushed through pipes to point of use for a minimum of 10 minutes).
3. It will be necessary to implement a long-term strategy for disinfection of the potable water system after emergency remediation.

XI. Long-term *Legionella* Control.

A. Water-management Experts: The facility's administration should consider re-evaluating the plan for the long-term disinfection and control of *Legionella* growth in the water system in conjunction with water-management experts.

B. Schematics: Diagraming the plumbing system and updating schematics as necessary is essential to identifying and eliminating high-risk areas of water stagnation and potential *Legionella* amplification, for example by discovering dead legs in the system.

C. Detectable Levels of *Legionella*: The facility should strive for the eradication of *Legionella* from the potable water system, as there is no known safe level of *Legionella*.

XII. DPH and Facility Collaboration on Remediation.

A. Facility Remediation-Plan Submission: Facility will submit a remediation plan to ACDC, EH, and HFID, as appropriate. The time frame for submission will be determined on a case-by-case basis.

B. Facility Remediation-Plan Details: Plan must include a method(s) of remediation and the agreed-upon schedule when remediation and sampling events are to occur
   1. If a third-party remediation company is hired by the facility, EH, in consultation with ACDC will work collaboratively with the company and the facility to ensure that the confirmed or potential source(s) of the cluster/outbreak is remediated.
   2. EH, in consultation with ACDC and, if appropriate, HFID will work with the facility to ensure that facility staff are conducting remediation activities as specified in the plan.
   3. If additional environmental testing is indicated, the facility will use a third-party ELITE certified laboratory [19] to test, which will provide all results to EH and ACDC
   4. Observation of re-sampling may be required by EH staff.

C. Feedback on Remediation Plan: Post-remediation results will be reviewed by EH, ACDC, and HFID as applicable, and in consultation with ACDC to determine if any additional and/or ongoing remediation is required.
   1. If further remediation with the facility is required (ACDC investigation is closed), EH will continue to work independently with the facility.
   2. If a third-party remediation company is hired by the facility, EH will work independently with the company until the closure of their investigation.
   3. EH will continue to provide ACDC all laboratory re-culturing test results, official communications and documents to the facility, and provide the final report sent to the facility when the EH investigation is closed.

XIII. Health and Safety Considerations.

A. Appropriate PPE Use for Environmental Assessment: During an environmental investigation of a facility associated with a cluster or outbreak of legionellosis, the team members conducting the environmental assessment and collecting samples may be exposed to pathogenic *Legionella* spp. Since the primary hazard to the person collecting samples is the inhalation of aerosolized water, preventing the aerosolization of water while samples are being collected is the best way to prevent exposure. OSHA's *Technical Manual on Legionnaires' Disease* [21] concludes that respiratory protection is necessary when there is a significant risk of exposure to high concentrations of contaminated aerosols, and appropriate PPE must be obtained prior to conducting the on-site assessment.

B. Minimizing Risk of Water Aerosolization: By turning off or disabling any pumps, fans, or other mechanical means that aerosolize water, the sample may be collected in the safest manner. First, these actions prevent inadvertent physical hazards from the pump, such as fan pinching, crushing an appendage, or electrical shock. Secondly, doing so minimizes water aerosolization during sample collection. The recommended approach to preventing inadvertent reactivation of the pump or fan is by using the appropriate lock-out equipment. If all measures are taken to prevent water aerosolization during sample collection, then respiratory protection should not be necessary. To avoid aerosolizing water from showers and faucets, the showerhead or faucet aerator should always be removed to collect water and to facilitate inspection and sampling of any biofilm on the back side of the shower head or faucet aerator.

Any incidents with potential exposure to an aerosol will be reported to the supervisor(s) of the exposed staff according to DPH policy.

C. Other Safety Measures: The DPH investigation team members shall take all appropriate safety measures, including safety briefings prior to conducting the assessment to avoid slips, trips, and falls, and electrical and overhead hazards. Non-slip insulated boots should be worn to guard against electrical shock from overhead electrical building components (light bulbs, electrical wiring, ungrounded systems, etc.). Goggles/safety glasses, and nitrile gloves should also be worn, and clothing should be without entrapment hazards, such as ties or loose clothing. When appropriate, DPH investigation team members should have the option to request a safety harness that can be attached to the access ladder to prevent accidental falls. Team members must also be aware of inadvertent exposures to cleaning or water-treatment chemicals often used and stored in and around cooling towers, as well as other environmental hazards such as asbestos, radiation, noise, rusty nails, and so on. Also, the team member should always be conscious of spiders, wasps, hornets, bees, and other stinging or biting insects that often live in and around rooftop mechanical equipment. Staff shall avoid working on any roof tops when there is a threat of thunderstorms. Any incidents with potential exposure to an aerosol will be reported to the supervisor(s) of the exposed staff according to DPH policy. The facility contact shall provide DPH with all necessary information to ensure DPH staff has familiarity with the facility areas to be inspected and be knowledgeable about access stairs and rooftop access ladders.

D. Other Environmental Assessment Tools: Useful CDC and ASHRAE environmental assessment tools include the following [15, 18, 20]:

1. Environmental Assessment of Water Systems
2. Environmental Testing Guidelines
3. Protocol for Sampling Environmental Sites for Legionella
4. Procedures for Collecting and Processing Environmental Samples
5. Potential Sampling Sites in Healthcare Facilities
6. De-contamination of Affected Water Systems: *ASHRAE Guideline 12-2000 -- Minimizing the Risk of Legionellosis Associated with Building Water Systems*.

(continued on next page)

XIV. Laboratory Testing Guidelines.

Laboratory Test	2009 Definition	Advantages	Disadvantages
Culture of respiratory secretions or tissue	Confirmed	■ Clinical & Environmental isolates can be compared ■ Detects all species & serogroups ■ 100% specific	■ Technically difficult ■ Slow (2–3 days to grow) ■ Sensitivity highly dependent on technical skill ■ May be affected by antibiotic treatment ■ Isolate must be grown on appropriate medium
Urinary antigen for *L. pneumophila* serogroup 1	Confirmed	■ > 99% specific for *L. pneumophiia* serogroup 1 (*Lp*1) ■ Rapid (same day)	■ Only tests for *Lp*1 ■ Limited utility when compared to environmental isolates
Serology — *L. pneumophila* serogroup 1 using validated reagents	Confirmed if ≥ 4-fold increase	■ Not affected by antibiotic treatment ■ 70–80% sensitive; > 90% specific	■ Must have paired serology specimens ■ 5–10% of the population has titer 1:>256 Single acute phase antibody titers of 1:>256 do not discriminate between cases of Legionnaires' disease and other causes of community-acquired pneumonia. ■ Not performed at LACPHL
Serology — species-specific antigen other than *L. pneumophila* serogroup 1	Suspect		
Serology — multiple species (pooled antigen)	Suspect		

			■ Some laboratories do not report serogroup in results
Detection of *Legionella* antigens or staining of the organism	Suspect — DFA, IHC, or other similar methods	■ Can be performed on pathologic specimens or culture isolate ■ 95% specific	■ 25–75% sensitive (on pathology specimens)
Validated nucleic acid assay	Suspect		

XV. Recommendations for Environmental-specimen Collection, Handling, and Transport.

A. Environmental Testing Background: Water Sampling Environmental samples from the facility or other sites are cultured, and the resulting *Legionella* isolates may be subtyped by monoclonal antibody typing (Mab) or DNA fragment-based methods such as pulsed-field gel electrophoresis (PFGE) or DNA sequencing methods, with the comparison to clinical isolates, if available.

B. Coordinating with PHL: Water Sampling Procedures
1. Collection and scope of test request are to be coordinated with Public Health Laboratory (PHL) prior to submission of samples.
2. ACDC will complete Public Health Laboratory Requisition Form H-3021 for each collected water sample [22]
3. Chain of Custody form and collection list(s) should accompany PHL test requisition form [24]

C. Selection of Testing Sites: Site and Water Sample Selection: See III Facility/Environmental Assessment, C. Environmental Health: Facility/Environmental Assessment, 4. Environmental Assessment Water Sampling. Also consider III Facility/Environmental Investigation Protocol, C. Environmental Health: Facility/Environmental Assessment, 1. Gathering Information and based on epidemiologic data gathered by the LPHN on the CDPH Legionellosis Case Report form [13]. Also, see CDC *Sampling procedure and potential sampling sites: Protocol for collecting environmental samples for Legionella culture during a cluster or outbreak investigation or when cases of disease may be associated with a facility* [17].

D. Selection of Swab Versus Water Sample Collection:
1. Swab Samples:
   a. Swabs should be collected prior to the collection of water from identified sites;
   b. After removal of showerheads, faucet aerators, or scum lines (fountains and recreational water facilities) insert a sterile polyester swab into the shower and sink outlets and rotate them against the interior surface to dislodge residue (approximately four times while moving swab upwards into the opening);
   c. Place swab in a 15 mL conical tube containing 3–5 ml of sample water taken from the same device to prevent drying.
2. Water samples:
   a. Use a 1-liter sterile Nalgene polypropylene bottle for collection;
   b. If water source has been chlorinated, add 0.5 mL of 0.1 N sodium thiosulfate to each liter sample bottle to neutralize residual chlorine;
   c. Pre-flush sample: After the swab sample has been collected, without flushing the line, collect a 1-liter (1L) hot/cold sample in an appropriate sterile water collection bottle. Use aseptic technique.
3. Flush sample: After flushing a line for a few minutes, collect a 1L hot and/or cold sample in a 1L sterile water collection bottle. Use aseptic technique.
4. Document the type of municipal water disinfectant used on the CDC Environmental Assessment of Water Systems Form [17]. Note the physical and chemical characteristics:
   a. Chlorine;
   b. Monochloramine;
   c. Other.
5. Record the pH, chlorine residuals levels, temperature (1st 50 mL then run 2–3 min for the second 50 mL) on the “Measurable Parameters” section of the CDC Environmental Assessment of Water Systems Form [18].

E. Considerations for Hot Water Storage Tanks:
   1. Immediately collect 1L of water into a sterile 1L bottle. This sample reflects the stagnant water in the supply pipe.
   2. Allow the water to flush for approximately 30 seconds and then collect another 1L of water into a sterile 1L bottle. This reflects the tank.
   3. Process both samples with direct plating.

F. Environmental-sample Handling and Transport Requirements:
   1. Submit samples to the PHL within 24 hours.
   2. Transport water samples and swabs, as soon after collection as possible, in insulated coolers at ambient temperature to the PHL (avoid exposing samples to temperature extremes):
      a. If unable to transport within 24 hours, store refrigerated at 4°C (39°F);
      b. Samples arriving at PHL and not processed same day of receipt should be at stored at 4°C (39°F) until initiation of testing.
   3. Investigators should refer to the CDC *Sampling Procedure and Potential Sample Sites* [17] and the OSHA *Technical Manual* [21] section on Legionnaires' Disease for further technical guidance on conducting environmental assessments and remediation efforts.

XVI. Recommendations for Patient-specimen Collection, Handling, and Transport.

A. Considerations for Patient-specimen Collection:
   1. Collect specimen from the site of infection (e.g. sputum).
   2. Collect and submit respiratory tract specimens in a sterile container.
   3. Induce sputum, using nebulized saline (can be used even though it has been shown that sodium ions may possibly reduce the recovery of *Legionella* spp.).
   4. Store specimens that are not processed within 30 minutes at 4°C (39°F). If a delay is > 48 hours, freeze specimen at −70°C (−94°F).

B. Acceptable PHL Specimens:
   1. Expectorated sputum:
      a. Evaluate specimen quality and select areas containing bloody or brown flecks or which are noticeably purulent;
      b. Sputum obtained by suction.
   2. Aspirates:
      a. Nasotracheal;
      b. Endotracheal;
      c. Transtracheal;
      d. Percutaneous Lung;
      e. Endobronchial.
   3. Bronchoscopy specimens:
      a. Bronchial washing;
      b. Lavage fluid;
      c. Brushing;
      d. Biopsy sample.
   4. Biopsy sample:
      a. Transbronchial;
      b. Open lung;
      c. Other (i.e., renal, liver, etc.).
   5. Fluid:
      a. CSF;

b. Pericardial;
c. Peritoneal;
d. Pleural.

6. Wound or abscess material: Acceptable if routine bacterial culture is negative.
7. Prosthetic heart valves.
8. Autopsy samples:
   a. Lung;
   b. Other tissue on rare occasions;
9. Paraffin-embedded tissue sections:
   a. Cut the tissue as thin as possible (≤ 4 µm);
   b. Fix sections at 58 ºC–60ºC (122°F–140°F) for 15 minutes;
   c. Deparaffinize by two passages through xylene followed by two passages each through absolute ethanol and distilled water;
   d. Urine: Acceptable for *Legionella* urinary antigen;
   e. Serum: Acceptable for antibody detection, but not routinely recommended for routine testing by CDC [25].

C. Unacceptable PHL Specimens:
1. Urine and serum are not the most reliable or sensitive specimens for *Legionella* culture and will not be tested.
2. Other specimen types not listed above.

D. Specimen Transport:
1. Transport specimens in sterile dry containers. If necessary, add a small amount of sterile, non-bacteriostatic, distilled water to prevent drying. *Do not add saline*, as sodium ions may inhibit the growth of some *Legionella.*
2. Ship refrigerated specimens on ice. Frozen specimens should be shipped on dry ice. Repeated freeze-thaw cycles should be avoided as this results in a decrease of viable organisms.
3. Specimens fixed in formalin or embedded in paraffin are satisfactory for DFA only.

E. Specimen Rejection:
1. Specimens will be rejected in the PHL for the following reasons:
   a. Case name discrepancy;
   b. Case file number discrepancy;
   c. No case name on the specimen.
2. Specimens to the PHL will not be rejected due to delay or mis-storage as *Legionella* has been recovered after the storage of specimens for several days at room temperature.
3. Completely dried out specimens in the PHL will be rehydrated and processed. Submitter will be notified of the condition of the specimen and decreased the likelihood of recovery of the organism.

F. PHL Required Equipment and Forms [22, 23, 24]:
   a. Los Angeles County Public Health Laboratory Requisition Form [22];
   b. Chain of Custody Form (the form used to document the handling of specimens by an authorized person(s) for the collection, transport, and delivery of specimens) [24];
   c. Environmental Sample Line List;
   d. Nalgene containers, 1L, sterile, with 0.5 mL sodium thiosulfate;
   e. Conical tubes, 15 mL, sterile, screw cap;
   f. Sterile swabs, Dacron with non-wooded shafts, flocked;
   g. Water, distilled, sterile;
   h. Black sharpie markers;

i. White adhesive labels;
j. Discard bags;
k. PPE and N95 mask where extensive aerosolization or other potential aerosolized biological health hazards may be present. Use of a selected combination of PPE will be dependent on the situation when collecting environmental samples;
l. Gloves.

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Appendix A: ACDC *Legionella* Action Plan and Checklist for Full Investigations

Pre-Site Visit

- Schedule teleconference (e.g. PHL, EH, HFID, DWP, etc.).
- For a community-associated investigation: Email outbreak summary to Chief, Deputy Director, with a CC to Area Medical Director. Write and obtain a signature for community posted letter (e.g. spa, pool, or non-healthcare facility).
- Prepare for site visit (discuss status/assign responsibilities).
- Recorders (photographer/scribe (take notes/log laboratory cultures).
- Prepare materials (chart abstraction tool, line list of positive cultures (staff/residents), picture log list, sign in sheet, camera, etc.).
- Prepare list of outstanding documents (e.g. staffing lists, med records).
- Arrange meeting time/place.
- Prepare site visit agenda.
- Prepare PPE.
- EH/Designated PH agency obtain and bring PHL supplies.
- Arrange for use of the LAC DPH vehicle.
- Arrange courier to bring a case and/or environmental isolates from the facility, if applicable.
- Open outbreak in LAC DPH reportable diseases surveillance system (currently vCMR), if applicable, and open in ACDC outbreak log.

Site Visit

- Entrance conference.
- Walkthrough inspection and collection of environmental specimens.
- Review Water-Management Plan (WMP), maintenance logs, policies and procedures.
- Chart review, if applicable.
- Map of facility.
- Exit conference.
- Public Notifications: A public notification (e.g. press statements, board notifications, postings, letters) will be issued if ACDC determines that such an action will reduce the public's risk for legionellosis or if recommended by DPH External Communications and confirmed by DPH leadership.

Post-Site Visit

- Summary of recommendations from site visit (includes all applicable PH agencies).
- Email summary to facility point of contact.
- Maintain laboratory results.
- Gather final letters from EH (Rec waters, Cross connections, Industrial Hygiene, Drinking water).
- Issue final report.

General ACDC Instructions

- DPH leadership will review and approve any public notification prior to sending it to the Office of Communication and Public Affairs for its release.

Appendix B: PHL Test Requisition Form

County of Los Angeles Public Health

COUNTY OF LOS ANGELES
DEPARTMENT OF PUBLIC HEALTH

PUBLIC HEALTH LABORATORY

TEST REQUISITION FORM

12750 ERICKSON AVENUE
DOWNEY, CA 90242
(562) 658-1300
FAX (562) 401-5999

California Certified Public Health Laboratory # 335637
CLIA # 05D1066369

Patient Name (Last, First)					Date/Time Received	Date/Time Reported
					Submitter	
Patient ID Number	Race	M	F	Date of Birth		
Specimen Source	Patient Location/Clinic			Date/Time Taken	Requesting Physician /Referring Laboratory	Submitter Accession #
Information for Viral Culture					**Information for Microbiological Exam**	
Date of onset:					PHN Code #:	
Suspected virus:					Outbreak #:	
Account Information					☐ Possible Child Abuse (Consult Laboratory)	
Submitter Account #:					☐ Possible Medico-Legal Case (Consult Laboratory)	

TEST REQUEST

- ☐ Aerobic Bacterial ID
- ☐ Aerobic Bacterial Culture
  - Specify: _____
- ☐ AFB, Amplified M. tuberculosis Direct Test
- ☐ AFB, Culture for Identification
- ☐ AFB, Smear "only"
- ☐ AFB, Smear, Culture and Susceptibility
- ☐ AFB Susceptibility
- ☐ Anaerobic Bacterial ID
- ☐ Anaerobic Bacterial Culture
  - Specify: _____
- ☐ Arbovirus AB Panel
- ☐ Blood Smear, Parasite Exam
- ☐ Bordetella Culture
- ☐ Bordetella PCR
- ☐ Campylobacter Culture
- ☐ C. trachomatis Culture
- ☐ C. trachomatis/N. gonorrhoeae Nucleic Acid Amplification Test
- ☐ C. botulinum – Toxin
- ☐ C. botulinum – Culture
- ☐ CMV Culture
- ☐ Cryptosporidium/Giardia DFA
- ☐ Cryptosporidium/Cyclospora/Isospora
- ☐ E. coli O157, Culture
- ☐ E. histolytica EIA
- ☐ Food – Specify: _____
- ☐ Fungal Culture and ID
- ☐ Fungal Culture ID
- ☐ Fungal ID, DNA Probe
  - ☐ Coccidioides immitis
  - ☐ Histoplasma capsulatum
- ☐ Hepatitis A Total Ab
- ☐ Hepatitis A IgM
- ☐ Hepatitis B Core Ab
- ☐ Hepatitis B Surface Ab
- ☐ Hepatitis B Surface Ag
- ☐ Hepatitis C Virus Ab
- ☐ HIV-1/2 Ab
- ☐ HIV-1 Resistance, Genotyping
- ☐ HIV-1 Viral Load, PCR
- ☐ HIV-1 Western Blot
- ☐ HSV 1/2 PCR
- ☐ HSV Culture
- ☐ HSV-2 IgG Ab
- ☐ Influenza Virus A/B PCR
- ☐ Lead, Blood
- ☐ M. tuberculosis, Molecular Detection of Drug Resistance
- ☐ M. tuberculosis, PCR
- ☐ Malaria Confirmation
- ☐ Microsporidium Exam
- ☐ N. gonorrhoeae Culture*
- ☐ N. gonorrhoeae - NAAT
- ☐ Ova and Parasite Exam
- ☐ Pinworm Prep.
- ☐ Quantiferon
- ☐ Rabies Ag, DFA
- ☐ Respiratory Virus Culture
- ☐ Respiratory Pathogen PCR Panel
- ☐ Rickettsial Ab Panel
- ☐ Rotavirus Ag Detection
- ☐ Salmonella Shigella Culture
- ☐ Shiga-like Toxin Screen
- ☐ Stool Culture – Specify _____
- ☐ Syphilis Reflex Panel
- ☐ T. vaginalis, NAAT
- ☐ Vibrio Culture
- ☐ Viral Culture Comprehensive
- ☐ Viral Identification
- ☐ West Nile Virus Ab
- ☐ Worm Identification
- ☐ Yersinia Culture
- ☐ Other: _____

H3021 (01-14)

*a – For Legal cases or surveillance