Laboratory Diagnosis of Legionellosis

The Laboratories Administration provides testing for clinical specimens and environmental samples

The outbreak of pneumonia in July 1976 that occurred among attendees of an American Legion convention in Philadelphia led to an investigation by the Centers for Disease Control and Prevention (CDC). This resulted in the identification of a causative agent, then referred to as *Legionella* bacterium. Since then, more than 48 species and 71 serogroups of *Legionella* bacteria have been identified. While more than half of these species/serogroups have been associated with human disease, *L. pneumophila*, accounts for approximately 90% of infections, with illness most frequently associated with serogroups 1, 4, and 6. *Legionella* can cause two clinically distinct syndromes: Legionnaires’ disease (LD) and Pontiac fever. LD is a multisystem illness, with mild to severe pneumonia, characterized by fever, cough, and progressive respiratory distress. In contrast, Pontiac fever is a milder febrile, self-limited influenza-like illness, without pneumonia, that occurs in clusters. The incubation period (the time in days from exposure to the onset of symptoms) is two to ten days for LD and one to three for Pontiac fever. In the United States, over 25,000 cases of the LD illness occur each year and cause more than 4,000 deaths. The fatality rate is approximately 15 percent, which is similar to other forms of pneumonia.

*Legionella* organisms are Gram-negative aerobic, motile, and nutritionally fastidious pleomorphic rods. The growth of the organism depends on the presence of L-cysteine and iron in special media. The organism has been isolated in natural aquatic habitats (freshwater streams and lakes, water reservoirs) and artificial sources (cooling towers, potable water)

(Continued on page 2)
distribution systems). Freshwater amoebae appear to be the natural reservoir for the organisms. Optimal growth temperature range is 28-40°C; organisms are dormant below 20°C and are killed at temperatures above 60°C.\(^7\),\(^8\)

LD is characterized as an “opportunistic” disease that attacks individuals who have an underlying illness or weakened immune system. Direct inhalation is the most common method of transmission, with aerosol-generating systems playing a crucial role.

Person-to-person transmission has not been documented. The highest incidence occurs during the warmer months, when air conditioning systems are used more frequently. Nosocomial acquisition likely occurs via aspiration, respiratory therapy equipment, or contaminated water. Transmission has also been linked to the use of humidifiers, nebulizers, and items that were rinsed with contaminated tap water. Increased colonization of \textit{Legionella} in man-made water environments has been linked to:

- Temperature of 25-42°C;
- Stagnation;
- Biofilm, deposits, scale, and sediment;
- Presence of certain free-living aquatic amoebae capable of supporting growth of \textit{Legionellae};
- Water pH 5.0 - 8.5;

Diagnosis

The clinical symptoms of LD are indistinguishable from the symptoms of other causes of pneumonia. Therefore laboratory diagnostic methods are needed for identification of \textit{Legionella}. The CDC defines a confirmed case of LD as a clinically compatible case that is confirmed by a laboratory. A confirmed case requires a physician's diagnosis of pneumonia based on a chest x-ray and positive laboratory test results. A laboratory test is necessary for confirmation because the symptoms and x-ray evidence of LD resemble those of other types of pneumonia.

The CDC laboratory criteria for diagnosis are:

1. Isolation of \textit{Legionella} bacteria from respiratory secretions, lung tissue, pleural fluid, or other normally sterile fluids;

2. Demonstration of a fourfold or greater rise in the reciprocal immunofluorescence antibody (IFA) titer to greater than or equal to 128 against \textit{Legionella pneumophila} serogroup 1 between paired acute-phase and convalescent-phase serum specimens;

3. Detection of \textit{L. pneumophila} serogroup 1 in respiratory secretions, lung tissue, or pleural fluid by direct fluorescent antibody testing; or

4. Demonstration of \textit{L. pneumophila} serogroup 1 antigens in urine by enzyme linked immunosorbent assay (ELISA).\(^6\)

(Continued on page 3)
(Continued from page 2)

**Laboratory Diagnosis of Legionellosis**

### Culture Isolation and Identification

Isolation by culture is the preferred diagnostic method. A number of factors limit the sensitivity of culture. Ideally, a specimen for culture should be taken before antibiotic treatment is initiated. Specimens of choice include respiratory secretions from sputum, bronchial alveolar lavage (BAL), and bronchial aspirates. *Legionella* survives poorly in respiratory secretions and immediate specimen processing of the culture is critical. Pretreatment of the specimen with heat or acidification can reduce other microflora. Identification of the number of *Legionella* spp. is carried out using buffered charcoal yeast extract (BCYE) agar containing L-cysteine. Supplements such as cefamandole, polymixin B, or anisomycin are used to reduce the competing microflora and increase selectivity of media. Suspected colonies of *Legionella* spp. are identified by growth on selective and differential media and confirmed by direct fluorescent assay (DFA) antibody testing.

Isolation of *Legionella* from environmental samples helps to identify the source in outbreak investigations. It also helps assess the effectiveness of remedial action. Proper environmental samples should be collected for microbial analysis. It is necessary to concentrate the sample and suppress the competing background flora during primary culture. Culture results provide useful information about the degree of *Legionella* contamination in a water system. It is difficult to interpret the colony count values for *Legionella* in relation to disease risk. Higher colony counts per unit volume result in a higher exposure, which could be related to a higher infection risk.

*L. pneumophila* serogroup 1 comprises a fairly heterogeneous group of organisms that accounts for most cases of legionellosis in the United States. *L. pneumophila* serogroup 1 can be divided into a number of subtypes by various phenotypic and genotypic techniques. These procedures are used to match environmental isolates with patient isolates obtained from outbreak investigations of Legionellosis. Because of the diversity within the *L. pneumophila* serogroup 1, clinical and environmental isolates must be matched by molecular techniques to adequately identify environmental sources of the disease. Pulsed field gel electrophoresis (PFGE) and arbitrarily primed polymerase chain reaction (AP-PCR) are able to discriminate within monoclonal subtype of *L. pneumophila* serogroup 1 and identify the source of the disease causing strain.

### Direct Fluorescent Assay (DFA)

Microscopic detection of *Legionella* in body fluids and tissue by DFA is a rapid method for identifying *Legionella* species. However, the sensitivity of DFA ranges from 25-75%. DFA specificity is 80-90%. Serological cross-reactions with non-*Legionella* bacteria have been reported against several *Legionella* species.

### Serological Testing

Indirect immunofluorescence assay (IFA), ELISA, and microagglutination have been used for detection of antibody for *L. pneumophila*. In the clinical setting, serology has had limited usefulness because to make a definitive clinical diagnosis a fourfold rise in antibody titer from specimens obtained three to six weeks apart is necessary. Patients with non-legionellosis pneumonia and bacteremia have been found to demonstrate falsely-positive *Legionella* serology results. Antibodies produced in response to infections with *Bacteroides fragilis*, *Chlamydia psittaci*, *Mycobacteria*, *Mycoplasmas*, *Campylobacters*, *Haemophilus influenzae*, *Coxiella burnetii*, *Rickettsia typhi*, *Proteus vulgaris OX19*, and *Pseudomonas aeruginosa* in cystic fibrosis patients also have been shown to be cross reactive in *Legionella* serological tests. Cross-reactions occur more frequently with non-*L. pneumophila* species. The sensitivity of *Legionella* serological assays ranges between 70-90% and specificity ranges between 50-99%.

### Urine Antigen Detection

The use of ELISA or the immunochromatographic assay for detecting *L. pneumophila* serogroup 1 antigen in urine is currently the most frequently used diagnostic test. Rapid testing of urine for *L. pneumophila* antigen is easy to perform and has excellent sensitivity (77-99%) and specificity (99-100%) for *L. pneumophila* serogroup 1. Urine antigen testing has led to the recognition of outbreaks of LD and allows for a rapid public health response. In addition, urine antigen testing permits early diagnosis and initiation of appropriate antibiotic therapy. However, it can only detect infections with *L. pneumophila* serogroup 1.
Laboratory Diagnosis of Legionellosis

Polymerase Chain Reaction (PCR)

The ribosomal RNA (rRNA) genes have been used for PCR assay targeting the *Legionella* genus, and the macrophage infectivity potentiator (*mip*) gene has been used as a specific assay for *L. pneumophila*. Currently, there is insufficient data to reliably estimate PCR sensitivity and specificity or to compare PCR to other methods. Several researchers have reported on the use of real-time PCR that utilizes hybridization probes to confirm the product identity for rapid detection of *Legionella* in clinical specimens.\(^\text{13}\)

Available testing

The Laboratories Administration provides *Legionella* testing for both clinical specimens and environmental samples. Isolation and identification of *Legionella* by culture, serum antibody testing, and urine antigen tests are performed for clinical diagnostic purposes. Isolation and identification of *Legionella* from environmental water samples are conducted exclusively in support of investigations of suspected outbreaks. PFGE is performed only when both clinical and environmental *Legionella* isolates are obtained and are epidemiologically linked to the same suspected outbreak of *Legionella*.

Questions concerning *Legionella* testing may be directed to laboratory scientists by calling 410-767-5837.

This article compiled by Dr. Leena Trivedi.

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M 83 1 BALTIMORE CITY
M 47 1 MONTGOMERY
F 65 1 OUT OF STATE
M 66 1 OUT OF STATE
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M 37 1 PRINCE GEORGE'S
M 71 1 WICOMICO
MYCOBACTERIUM GORDONAE
M 52 1 ANNE ARUNDEL
F 61 1 BALTIMORE
M 31 2 HARFORD
F 68 1 MONTGOMERY
F 84 1 MONTGOMERY
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M 47 1 PRINCE GEORGE'S
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MYCOBACTERIUM MARINUM
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MYCOBACTERIUM SZULGAI
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MYCOBACTERIUM TERRAE
M 74 1 BALTIMORE CITY
MYCOBACTERIUM TUBERCULOSIS
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F 64 1 BALTIMORE
M 41 1 BALTIMORE
M 63 1 BALTIMORE
M 58 1 HARFORD
M 39 2 HOWARD
F 68 1 MONTGOMERY
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M 53 1 MONTGERY
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M 84 1 PRINCE GEORGE'S
U 0 1 UNKNOWN
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M 63 1 BALTIMORE
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M 58 2 HARFORD
M 39 3 HOWARD
M 16 3 MONTGOMERY
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M 53 3 MONTGOMERY
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M 47 1 OUT OF STATE
M 61 1 OUT OF STATE
U 87 1 OUT OF STATE
F 36 3 PRINCE GEORGE'S

TOTAL 429
### Parasyitology

**Genus/Species**  | **# Jurisdiction**
--- | ---
Blastoscytis Hominis  | 1 Frederick, 3 Prince George's
Dientamoeba Fragilis  | 3 Howard
Endolimax Nana  | 3 Prince George's, 4 Montgomery, 1 Howard
Entamoeba coli  | 1 Howard, 1 Montgomery, 1 Prince George’s, 4 Baltimore City, 1 Prince George’s, 3 Montgomery, 4 Prince George’s, 1 Howard, 1 Frederick, 1 Montgomery, 1 Montgomery, 1 Howard, 1 Frederick, 1 Prince George’s, 2 Montgomery, 3 Prince George’s
Entamoeba hartmanni  | 2 Baltimore City, 1 Montgomery, 1 Frederick, 3 Montgomery, 1 Carroll
Enterobius vermicularis  | 2 Talbot, 1 Carroll, 1 Frederick
Giardia lamblia  | 2 Prince George’s, 1 Montgomery, 1 Montgomery
Iodamoeba bütschlii  | 1 Harford
Larvae  | 1 Harford

**Total**  | 145

### Viral Polymerase Chain Reaction (PCR)

**Isolate**  | **Sex** | **Age** | **Jurisdiction**
--- | --- | --- | ---
**Adenovirus**  | 1 F 45 | 5 Prince George’s
 | 1 M 24 | 1 Prince George’s
 | 1 M 63 | 2 Prince George’s
 | 1 M 56 | 5 Somerset
 | 1 M 56 | 3 Wicomico

**Rapidly Growing Mycobacteria**  | 1 Frederick, 1 Prince George’s

**Scotochromogenic Mycobacteria**  | 1 Frederick, 1 Prince George’s

**Non-photochromogenic Mycobacteria**  | 1 Frederick, 1 Prince George’s

### Water Microbiology

**Commmunity**  | **# Tested** | **# Non-Compliant**
--- | --- | ---
1  | 0
181  | 28
**Total**  | 182  | 28

### Food Protection

**Food**

**Number of Samples**  | 40
**Notable Pathogens:**
- Campylobacter sp.  | 6
- Enterococcus  | 33
- E. coli  | 13
- MRSA  | 24
- Salmonella sp.  | 3
- VRE  | 6

**Crabmeat**

**Number of Samples**  | 0
**Exceeding Standards**  | 0
**Notable Pathogens:**
- Listeria innocua  | 0

**Shellfish**

**Number of Samples**  | 0
**Exceeding Standards**  | 0

**Total Standards Exceeded**  | 0

**Shellfish Growing Waters**

**Number of Samples**  | 97

**Total Number of Samples**  | 137

**Standards**

1. **Crabmeat Fresh**
   - Escherichia coli at < 36 MPN/100 grams

2. **Shellfish**
   - Fecal coliforms at < 230 MPN/100 grams
   - Standard plate count at < 500,000 per gram

### Virus Isolation

**Isolate**  | **Sex** | **Age** | **Jurisdiction**
--- | --- | --- | ---
**Adenovirus**  | 1 F 45 | 5 Prince George’s
 | 1 M 24 | 1 Prince George’s
 | 1 M 63 | 2 Prince George’s
 | 1 M 56 | 5 Somerset
 | 1 M 56 | 3 Wicomico

**Herpes Simplex Virus Type 1**

**Herpes Simplex Virus Type 2**

**Influenza A Virus**

**Influenza B Virus**

**Total**  | 31

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Critical Link • www.dhmh.state.md.us/labs/html/critical-link.html • April 2011 • Vol. 15, No. 4
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Critical Link • www.dhmh.state.md.us/labs/html/critical-link.html • April 2011 • Vol. 15, No. 4
### CHLAMYDIOPHILIA PSITTACI (CHLAMYDIA)

**REPORTED QUARTERLY**

**NO REPORT THIS MONTH**

### RABIES

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**SUBTOTAL** 1,483 17

### VIRAL HEPATITIS

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**SUBTOTAL** 1 0

### HEPATITIS C

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**SUBTOTAL** 972 114

### TOTALS

**TOTAL SPECIMENS** 2,456 131

### CD4 FLOW CYTOMETRY WORKLOAD

**REPORTED QUARTERLY**

**NO REPORT THIS MONTH**
### Newborn & Childhood Screening
#### Presumptive Positives

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<th>Disorders</th>
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<td>Homocystinuria</td>
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<td>Galactosemia</td>
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<td>Biotinidase deficiency</td>
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<tr>
<td>Hypothyroidism</td>
<td>62</td>
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<tr>
<td>Hemoglobin - disease</td>
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<tr>
<td>Hemoglobin - benign</td>
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<tr>
<td>Congenital adrenal hyperplasia (CAH)</td>
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<td>Cystic fibrosis</td>
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<td>Fatty acid oxidations</td>
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<td>Organic acidemias</td>
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<tr>
<td>Acylcarnitine - borderline</td>
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<td>Acylcarnitine - others</td>
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#### Monthly Totals

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<tr>
<td># of specimens screened</td>
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<tr>
<td>Number of tests</td>
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<td>% Unsatisfactory specimens</td>
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#### 2011 Year-to-Date Confirmed Cases

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<td>Hypothyroidism - primary</td>
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<td>Other Hypothyroidism</td>
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<td>TBG deficiency</td>
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<tr>
<td>Sickle cell disease - SS</td>
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<td>Biotinidase deficiency - Carrier</td>
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<td>Cystic fibrosis</td>
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<td>Galactosemia - variant - DG</td>
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### Environmental Chemistry

#### Sample Types

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#### Asbestos

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#### Air Quality

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#### Radiation

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#### Drinking Water

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#### Pesticides & PCBs

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#### Food Chemistry

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#### Total

|                         | 30            | 884    |
### VIRAL LOAD SPECIMENS

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### HIV ANTIBODY SCREENING

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<th>% EIA POSITIVE</th>
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<td>HEALTH DEPT, NON-STD, FAMILY PLANNING</td>
<td>314</td>
<td>1</td>
<td>0.32%</td>
<td>1</td>
<td>100.00%</td>
</tr>
<tr>
<td>HEALTH DEPT, NON-STD, OB/GYN</td>
<td>93</td>
<td>1</td>
<td>1.08%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>HEALTH DEPT, NON-STD, OTHER</td>
<td>442</td>
<td>32</td>
<td>7.24%</td>
<td>31</td>
<td>96.88%</td>
</tr>
<tr>
<td>HEALTH DEPT, STD CLINICS</td>
<td>991</td>
<td>14</td>
<td>1.41%</td>
<td>12</td>
<td>85.71%</td>
</tr>
<tr>
<td>HOSPITAL, OTHER</td>
<td>114</td>
<td>6</td>
<td>5.26%</td>
<td>5</td>
<td>83.33%</td>
</tr>
<tr>
<td>HOSPITAL, PUBLIC</td>
<td>30</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>LABORATORIES (NON-HOSPITAL)</td>
<td>314</td>
<td>11</td>
<td>3.50%</td>
<td>7</td>
<td>63.64%</td>
</tr>
<tr>
<td>PEDIATRIC - CHILD HEALTH</td>
<td>7</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>PRIVATE PHYSICIANS</td>
<td>2</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>PRIVATE STUDENT HEALTH CTRS</td>
<td>41</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>PUBLIC STUDENT HEALTH CTRS</td>
<td>227</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td>3,213</td>
<td>90</td>
<td>2.80%</td>
<td>79</td>
<td>87.78%</td>
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</tbody>
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