Overview

Useful For
Sensitive and rapid diagnosis of pneumonia caused by Legionella species

The assay is not recommended as a test of cure because bacteria nucleic acids may persist after successful treatment.

Method Name
Rapid Polymerase Chain Reaction (PCR)

NY State Available
Yes

Specimen

Specimen Type
Varies

Necessary Information
Specimen source is required.

Specimen Required
The high sensitivity of amplification by PCR requires the specimen to be processed in an environment in which contamination of the specimen by Legionella DNA is unlikely.

Specimen Type: Respiratory

Sources: Sputum, tracheal secretions/aspirates, transtracheal aspirate, bronchial washing/aspirate, bronchoalveolar lavage, lung fluid or pleural fluid

Container/Tube: Sterile container

Specimen Volume: 1 mL

Specimen Type: Fresh tissue or biopsy

Sources: Lung

Container/Tube: Sterile container

Specimen Volume: Entire collection

Forms
If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume
Fluid: 0.5 mL
Tissue: 5 mm(3)
Reject Due To

| Tissue/Other | Tissue in formalin, formaldehyde, or acetone; formalin-fixed paraffin-embedded (FFPE) block |

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varies</td>
<td>Refrigerated (preferred)</td>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>7 days</td>
<td></td>
</tr>
</tbody>
</table>

Clinical and Interpretive

Clinical Information

Legionnaires disease was first recognized during a pneumonia outbreak at the Legionnaires convention in Philadelphia in 1976. Investigators with the CDC isolated a novel, gram-negative bacillus, later named *Legionella pneumophila*. It is now widely recognized that *L pneumophila* (and other members of the genus Legionella) cause Legionnaires disease.

Reference Values

Not applicable

Interpretation

A positive PCR result for the presence of a specific sequence found within the *Legionella* 5S rRNA gene indicates the presence of a *Legionella* species DNA, which may be due to *Legionella* infection or environmental/water *Legionella* DNA in the specimen.

A negative PCR result indicates the absence of detectable *Legionella* DNA in the specimen, but does not rule-out legionellosis as false-negative results may occur due to inhibition of PCR, sequence variability underlying the primers and probes, or the presence of *Legionella* species in quantities less than the limit of detection of the assay.

Cautions

This assay does not differentiate between the *Legionella* species. False-positive results are theoretically possible if patient specimens are contaminated with *Legionella* DNA, which may occur since *Legionella* species are environmental organisms present in aquatic environments. The following uncommonly encountered species of *Legionella* are not detected by this assay: *Legionella anisa*, *L feeleii*, *L maceachernii*, *L parisiensis*, and *L sainthelensi*.

Supportive Data

In a Mayo Clinic study, 153 archived respiratory specimens previously tested for *Legionella* species by direct fluorescence antibody (DFA) testing were extracted and tested using this PCR method. The PCR assay was 100% sensitive and 99.3% specific, in comparison to DFA. Additionally, 30 lung tissues and 30 pleural fluids were spiked with 3 of the most commonly isolated *Legionella* species. Spiking studies showed similar analytical sensitivity for PCR and the DFA method. The analytical sensitivity was less than 50 targets/20 microliter reaction. No cross-reactivity was observed when tested on a panel of respiratory pathogens or normal flora bacteria of the upper respiratory tract. Thirteen serogroups of *Legionella pneumophila* (*L pneumophila* serogroups 1-12, 15/16) and 9 additional *Legionella* species (*Florribacter [Legionella] bozemanae*, *Florribacter [Legionella] dumoffii*, *L gormanii*, *L


**Performance**

**Method Description**

This method employs a target-specific detection system using fluorescent resonance energy transfer (FRET) hybridization probes designed for a specific sequence found within the *Legionella* 5S rRNA gene. The LightCycler instrument amplifies and monitors target nucleic acid sequences by fluorescence during PCR cycling. This is an automated PCR system that can rapidly detect amplified product development through stringent air-controlled temperature cycling and capillary cuvettes. The detection of amplified products is based on the FRET principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3’ end is excited by an external light source, which emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Ted 640, on the 5’ end. The acceptor fluorophore then emits light of a different wavelength that can be measured with a signal that is proportional to the amount of specific PCR product. The detection process is completed in <1 hour using a closed tube system.(Cunningham SA, Sloan LM, Uhl JA, et al: Validation of a real-time PCR assay for the detection of *Legionella* species in respiratory samples. Abstracts of the Annual Meeting of the Association for Molecular Pathology, 2009 General Meeting, Kissimmee, FL, Nov. 19-22, 2009)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday through Sunday

**Analytic Time**

3 days

**Specimen Retention Time**

7 days

**Performing Laboratory Location**

Rochester

**Fees and Codes**
Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

87801

LOINC® Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEGRP</td>
<td>Legionella PCR</td>
<td>5020-3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRC57</td>
<td>Specimen Source</td>
<td>31208-2</td>
</tr>
<tr>
<td>29515</td>
<td>Result</td>
<td>5020-3</td>
</tr>
</tbody>
</table>