MAYO CLINIC LABORATORIES

Test Definition: LEGRP

Legionella species, Molecular Detection, PCR, Varies

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 polymerase chain reaction requires the specimen to be processed in an environment in which contamination of the specimen by *Legionella* DNA is unlikely.

Specimen Type: Lower respiratory
Sources: Bronchoalveolar lavage, bronchial aspirate/brushing/lavage/washing, tracheal/endotracheal secretions/aspirate, sputum
Container/Tube: Sterile container
Specimen Volume: 1 mL

Specimen Type: Fresh tissue or biopsy
Sources: Lung, pleura, heart valve, pericardium
Container/Tube: Sterile container
Specimen Volume: Entire collection or 5 mm(3) - approximately the size of a pencil eraser
Collection Instructions: Aseptically collect a 1 to 2 cm(3) piece of tissue whenever possible

Specimen type: Fluid
Sources: Pericardial, pleural, chest, chest tube drainage, thoracentesis, empyema
Container/Tube: Sterile container

Specimen Volume: 1 mL

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

Specimen Minimum Volume
See Specimen Required

Reject Due To

Tissue in formalin, formaldehyde, or acetone Formalin-fixed paraffin-embedded (FFPE) block	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)	7 days	
	Frozen	7 days	

Clinical & Interpretive

Clinical Information
Legionnaires disease was first recognized during a pneumonia outbreak at the Legionnaires convention in Philadelphia in 1976. Investigators with the Centers for Disease Control and Prevention 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 polymerase chain reaction (PCR) result for the presence of a specific sequence found within the *Legionella* 5S ribosomal RNA 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*, *Legionella feeleii*, *Legionella maceachernii*, *Legionella parisiensis*, and *Legionella 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 polymerase chain reaction (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 frequently 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 (*Fluoribacter [Legionella] bozemanae*, *Fluoribacter* [*Legionella] dumoffii*, *Legionella gormanii*, *Legionella jordanis*, *egionella longbeachae*, *Legionella micdadei*, *Legionella oakridgensis*, *Legionella hackeliae*, and *Legionella wadsworthii*) included in the panel were detected with the PCR method.

Clinical Reference

1. Hayden RT, Uhl JR, Qian X, et al: Direct detection of *Legionella* species from bronchoalveolar lavage and open lung biopsy specimens: comparison of LightCycler PCR, in situ hybridization, direct fluorescence antigen detection, and culture. J Clin Microbiol. 2001;39(7):2618-2626. doi: 10.1128/JCM.39.7.2618-2626.2001.
2. Diederen BM, Kluytmans JA, Vandenbroucke-Grauls CM, Peeters MF: Utility of real-time PCR for diagnosis of Legionnaires' disease in routine clinical practice. J Clin Microbiol. 2008;46(2):671-677. doi: 10.1128/JCM.01196-07.
3. MacDonell MT, Colwell RR: The nucleotide sequence of the 5S rRNA from *Legionella pneumophila*. Nucleic Acids Res. 1987;15(3):1335. doi: 10.1093/nar/15.3.1335.
4. Rucinski SL, Murphy MP, Kies KD, Cunningham SA, Schuetz AN, Patel R: Eight years of clinical Legionella PCR testing illustrates a seasonal pattern. J Infect Dis. 2018 Jul 13;218(4):669-670. doi: 10.1093/infdis/jiy201.

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 ribosomal RNA gene. The LightCycler (LC) instrument amplifies and monitors target nucleic acid sequences by fluorescence during polymerase chain rection (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 probed 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 under an 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, Nov. 19-22, 2009; Rucinski SL, Murphy MP, Kies KD, Cunningham SA, Schuetz AN, Patel R. Eight years of clinical Legionella PCR testing illustrates a seasonal pattern. J Infect Dis. 2018 Jul 13;218(4):669-670. doi: 10.1093/infdis/jiy201)

PDF Report
No

Day(s) Performed
Monday through Sunday

Report Available
3 days

Specimen Retention Time
7 days

Performing Laboratory Location
Rochester

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
87801

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
LEGRP	Legionella PCR	5020-3

Result ID	Test Result Name	Result LOINC® Value
SRC57	Specimen Source	31208-2

29515	Legionella PCR, Result	5020-3