

Legionella pneumophila by Real-Time PCR

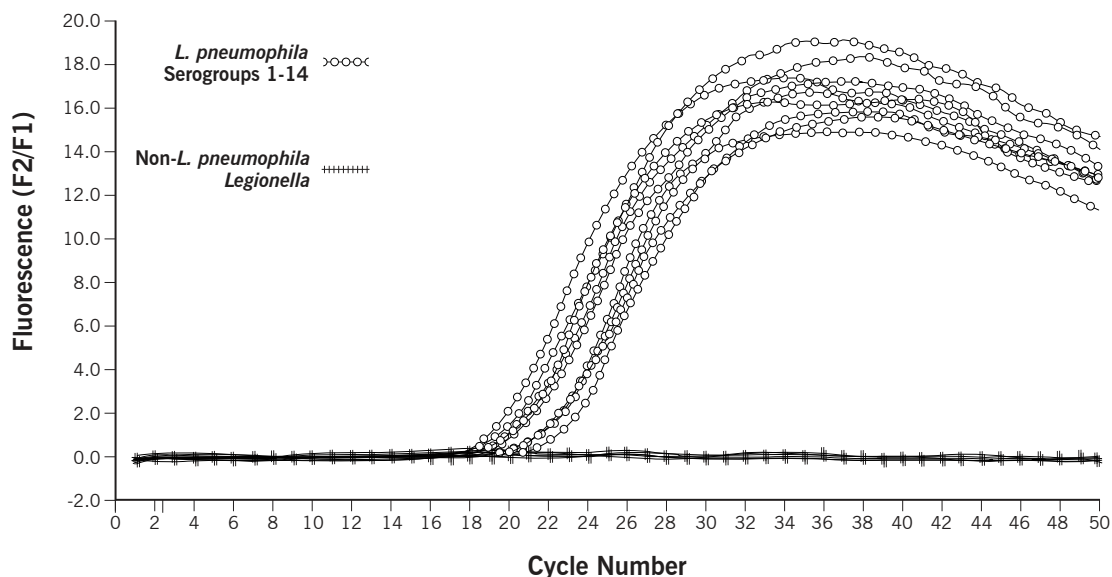
Background Information

Legionella pneumophila is the most common pathogenic species of the 42 recognized *Legionella* species and is associated with significant mortality in elderly patients and those with severe underlying disease. Diagnostic delay also may result in increased mortality. Target sequences within the genes that encode the 5S and 16S ribosomal subunits, as well as the *mip* (macrophage infectivity potentiator) gene, in conjunction with real-time PCR are useful for the detection of the *Legionella* genus and, specifically, the species *L. pneumophila*.

The *mip* gene, which encodes a 24-kDa protein virulence factor that facilitates the entry of legionellae into amoebae and macrophages, has sufficient sequence variability between the *Legionella* species to allow for the specific detection of *L. pneumophila* by real-time PCR. This assay has 100% specificity and sensitivity.

Clinical Information

Multiple laboratory methods should be employed to ensure the diagnosis of Legionnaire's disease (LD), a bacterial pneumonia caused by *L. pneumophila* (90% of cases) or other *Legionella* species. Molecular methods are more sensitive than culture for the diagnosis of LD. The PCR assay performed at Cleveland Clinic will not detect disease caused by *Legionella* species other than *L. pneumophila*. Culture for *Legionella* species from respiratory sites is a sensitive (~80-90%) method for diagnosing severe, untreated disease, but insensitive (~20%) for the diagnosis of mild disease. Specimens from nonrespiratory sites should not be submitted for *Legionella* culture unless there is a high index of clinical suspicion to support the request. Urine antigen assays for *L. pneumophila* serogroup 1 will be positive in ~90-95% of patients with severe disease due to the Pontiac monoclonal subtype of serogroup 1, but positive in only 50% of these patients with mild disease. The urine antigen assay is unreliable for the diagnosis of severe LD caused by *L. pneumophila* other than serogroup 1 or a different *Legionella* species (detects less than 5-40% of cases).



Interpretation

PCR results are reported qualitatively as positive or negative for *Legionella pneumophila*.

Limitations of the Assay

This assay does not contain an internal amplification control. Instead, the false-negative rate, which to date has been 0%, is monitored according to College of American Pathologists guidelines. *Legionella* species other than *L. pneumophila* infrequently cause legionellosis. This assay only detects *L. pneumophila*. The implementation of this test was intended to replace the less sensitive direct immunofluorescence assay, and it is intended to be used in conjunction with culture.

Methodology

The LightCycler FastStart DNA Master Hybridization Probe Kit (Roche Diagnostics, Indianapolis, Ind.) is used in conjunction with species-specific *Legionella pneumophila* primers and probes. The PCR is performed on the LightCycler system (Roche). Qualitative positive and negative results are determined based on analysis of the amplification curves and post-amplification melt curve.

Suggested Reading

Wilson DA, Yen-Lieberman B, Reischl U, Gordon SM, and Procop GW. Detection of *Legionella pneumophila* by Real-Time PCR of the *mip* Gene. *J Clin Microbiol* 2003;41:3327-3330.

Test Overview

Test Name	<i>Legionella pneumophila</i> PCR
Reference Range	No <i>Legionella pneumophila</i> detected
Specimen Requirements	1 ml induced sputum, tracheal aspirate, or bronchoalveolar lavage fluid in sterile container
Stability	Ambient: 2 hours; Frozen: 1 week; Refrigerated: 48 hours
Ordering Mnemonic	LEGPCR
Billing Code	77916
CPT Code	87541

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