

LABORATORY DIAGNOSIS OF LEGIONELLOSIS

INTRODUCTION

Legionellosis is a notifiable disease caused by infections with *Legionella* organisms. The infection is an important cause of adult pneumonia and must be actively considered in both community acquired and nosocomial pneumonia. Since legionellosis has no clinical features that distinguish it from other respiratory diseases, it can only be confirmed with confidence by the appropriate laboratory testing. The early recognition of cases is also paramount to the effective implementation of control measures in outbreak situations.

THE AGENT

There are 40 different legionella species of which 19 are known to be pathogenic. Some species are classified further into serogroups on the basis of their antigenic differences. All species are widespread in aquatic and damp soil environments. They can persist for months in potable water systems, damp soils and composted vegetative matter. In their natural environment they have a considerable capacity to survive and are facultative intracellular parasites. The infections they cause in humans are opportunistic, resulting from a combination of factors including contact with an infected environmental source and a predisposition to the disease. Infection is primarily caused by inhalation of aerosols containing legionella or the aspiration of legionella-contaminated water. There is no person-to-person spread. There is no significant difference in the virulence or sequelae, regardless of the identity of infecting legionella strain. Legionella are weakly staining Gram-negative rods 0.3-0.9 μm wide and 2-20 μm long. They are poorly motile with one or two polar flagella although non-motile strains exist. There is also no subclinical carriage of legionella. Under laboratory conditions they are slow growing, fastidious organisms, requiring L-cysteine and iron salts for growth.

THE DISEASE

Legionella infections can be classified into 4 categories: (i) subclinical infection, (ii) non-pneumonic disease, (iii) pneumonia, and (iv) extrapulmonary inflammatory disease. The two most common clinical manifestations of legionellosis are:

1. **Legionnaires' Disease:** the incubation period is usually 2-10 days with less abrupt onset of the pneumonia than is typically seen with pneumonic episodes caused by other organisms. There is often no sign of respiratory involvement in the early stages of the disease with symptoms including fever, rigors, myalgias and headache. A non-productive cough that follows 4 to 6 days later may be minimal with small amounts of purulent sputum produced. Sometimes non-respiratory symptoms such as nausea, vomiting, diarrhoea and/or delirium may dominate the clinical picture. Progression of the lung infection may be rapid from this point with greater production of purulent sputa.
2. **Pontiac Fever:** a self-limiting, influenza-like, nonpneumonic illness with a short incubation period (4 hours to 3 days). Symptoms usually include fever, malaise, myalgias, and cough. There is an absence of pulmonary infiltrates with spontaneous resolution in 2-5 days.

Note: The manifestation of subclinical infection, as demonstrated by a legionella antibody seroconversion without clinical symptoms, may be a contributing factor to the high antibody levels frequently seen in the general population in legionella sero-prevalence studies. The background antibody titres seen in the New Zealand population can be as high as 128 and 256 in 10-20% of the population, making diagnosis of legionellosis on the basis of a single serum sample impractical.

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TESTS ROUTINELY CARRIED OUT AT ESR FOR CONFIRMATION OF LEGIONELLOSIS

- Culture for Legionella from clinical specimens
- Confirmation of presumptive Legionella culture isolates referred by other labs
- Examination of clinical specimens for the presence of Legionella by PCR &/or DFAT
- Identification of Legionella culture isolates to species & serogroup level
- Examination of paired sera for the presence of Legionella-specific antibodies by IFAT
- PCR-based detection of Legionella in clinical specimens

COLLECTION OF APPROPRIATE CLINICAL SAMPLES

Samples for Legionella culture

- Any invasive lower respiratory tract specimen including the following:

- Bronchoalveolar lavage fluid
- Endotracheal aspirates (any bronchial or tracheal aspirate or brushing)
- Transtracheal aspirates (TTA)

Note: Avoid collecting lower respiratory tract samples with sodium salt-based buffers as these have been shown to be deleterious for legionella culture. Instead, use potassium salt-based buffers.

- Other respiratory tract specimen including the following:

- Deep throat swab – from trachea (collect specimen with dry cotton bud swab and place in sealed container with sufficient sputa to prevent drying in transport. Alternatively, add 0.5 to 1.0 mL of sterile and 0.1µm-filtered water to the swab. **NB: do not add any other solutions, especially saline).**
- Expecterated sputum (especially following TTA collection)

Note: Repeat sampling of any respiratory tract samples is useful to increase the chance of recovering legionella since the organism may be initially absent in the sample or in very low numbers.

Note: Ideally sampling should be taken prior to initiation of antibiotic treatment, although samples can still be culture positive after antibiotic treatment has begun.

Note: Pleural fluids rarely yield positive results. Upper respiratory tract samples rarely yield positive results.

- Venous blood specimens

- At least 5 mL of blood in sterile EDTA tube (not heparin)
- Conventional aerobic blood-culture bottles

- Biopsy and post-mortem specimens (freshly collected):

- Endocarditis with negative blood culture
- Gram-negative bacilli infections that can't be cultured by standard methods

Samples for Legionella PCR

- All samples listed for legionella culture are acceptable for legionella PCR, as well as:
 - 5 mL serum collected into SST tube and centrifuged prior to sending

Samples for Legionella DFA testing

- All samples listed for legionella culture are acceptable for legionella DFA, as well as:
 - Paraffin-embedded biopsy and post-mortem tissues
 - Fresh biopsy and post-mortem tissues

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COLLECTION OF APPROPRIATE CLINICAL SAMPLES (continued)

Samples for Legionella antibody serology

- Paired sera only (for retrospective diagnosis)
 - An acute-phase serum is taken within the first week of onset and stored frozen. A convalescent-phase serum is taken 3 weeks later with both tested in parallel.
 - A follow-up serum taken at 6 weeks if seroconversion has not occurred or first test is negative. If subsequent antibody testing is negative, a further sample should be provided at 90 days post-onset as maximum sensitivity for seroconversion occurs at 90 days for some patients.
- Single serum
 - Not considered a valid test sample unless done as an adjunct to Legionella culture. Single serum samples should be retained and tested in parallel with convalescent serum.

TRANSPORTATION OF ALL SAMPLES

- All clinical specimens must be sent chilled and without delay to the Legionella Reference Laboratory.
- Ideally, send specimens to arrive at the lab within 24 hours of their collection.
- Please note: Do not send specimens overnight Friday as no deliveries are made to ESR, Kenepuru Science Centre, Porirua during the weekend.
- Culture isolates must be sent in a sealed container at ambient temperature on solid media.
- Please ensure a completed ESR test request form with clinical details accompanies each specimen.
 - **DO NOT FREEZE SAMPLES FOR LEGIONELLA CULTURE**
 - **Avoid repeat freeze/thaw cycles of serum specimens if sent frozen**

CONTACT DETAILS

Delivery Address

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