



## ***Molecular detection of *Leptospira interrogans* DNA***

Pathogenic *Leptospira interrogans* are spirochaete bacteria with over 200 serotypes known to cause the disease Leptospirosis. Leptospirosis is the most widespread global zoonosis known to occur in all continents, with the exception of Antarctica<sup>1</sup>. *Leptospira sp.* are Gram-negative, flexible and helical bacteria. The details of the mechanisms of virulence and host survival tactics used by *Leptospira* are currently unknown. Human disease is generally contracted through contact with the urine or faeces of infected host animals, including cattle, dogs, and marsupials<sup>2</sup>. This contact typically occurs through broken or abraded skin and mucosal surfaces; however, it may occasionally occur through the inhalation of droplets of infected urine or contaminated water. Those at higher risk of contracting Leptospirosis include farm or sewage workers and individuals who undertake recreational water sports activities. Additionally, the disease is emerging in travellers to tropical regions, especially regions of South East Asia where rice paddy field workers are at regular occupational risk.

### **Signs and Symptoms**

The spectrum of symptoms of Leptospirosis infection is extremely broad; patients can present with generalised and mild symptoms or more severe forms of disease such as Weil's<sup>3</sup>. Leptospirosis is a biphasic disease with infection initially presenting as flu-like symptoms within the first week, followed by the immune phase. It is during this immune phase that most complications with infection occur. The infection has a 7 to 12-day incubation phase, followed by Leptospiraemia, characterised by pyrexia, myalgia and rigors that runs for 4 to 7 days. This is then followed by the immune phase, with approximately 10% of patients going on to develop Weil's disease and associated haemorrhagic complications during this time. *Leptospira* that are typically excreted in the urine during the convalescent phase can alternatively be localised within tissues. The subsequent infection is more severe, presented in its most classic form as Weil's disease. Subclinical infection typically occurs in those in high risk occupations.

<sup>1</sup> Adler, B. and de la Pena Moctezuma. 2011. *Leptospira* and leptospirosis. *Veterinary Microbiology*, vol. 140, no. 3-4, pp. 287-296.

<sup>2</sup> Gomes-Solecki, M., Santecchia, I. and Werts, C. 2017. Animal models of Leptospirosis: of mice and hamsters. *Frontiers in Immunology*, vol. 8, no. 58.

<sup>3</sup> Levett, P. 2001. Leptospirosis. *Clinical Microbiology Reviews*, vol. 14, no. 2, pp. 296-326.

## Diagnosis

Diagnosis can be difficult, due to the non-specific symptoms that can mimic the clinical manifestations of conventional flu<sup>4</sup>, hence it is frequently misdiagnosed. Abnormal liver and kidney function are typical of Leptospirosis, with increased levels of creatine kinase also suggesting infection. *Leptospira sp.* can be identified from the blood and cerebrospinal fluid (CSF) during the immune phase, approximately two weeks after infection, and from the blood specifically less than 48 hours post onset of jaundice. Urine is also an appropriate sample type, as the bacteria can be shed in the urine.

Serology tests can also be performed, which involve performing microscopic agglutination tests (MAT) with live *Leptospira*. Tests may also be performed with killed bacteria, although this has a lowered sensitivity. These tests can be relatively specific to serovars; however, a large number of antigens must be tested, and cross reactions can occur. The *Leptospira* Polymerase Chain Reaction (PCR) assay, on the other hand, is sensitive and able to distinguish between different bacterial species. It also allows for early diagnosis and organisms can also be detected after antibiotic treatment has commenced. Early diagnosis is beneficial as samples can be sent when patients are exhibiting non-specific symptoms in the initial phases of infection, allowing exact treatments to commence promptly.

## Our Assay

At Micropathology Ltd., we use a nested PCR assay for the detection of *Leptospira interrogans* DNA, using standard block thermocyclers with subsequent visualisation using agarose gel electrophoresis or melt curve analysis using LightCycler 480s. UKAS accredited sample types for this assay include EDTA whole blood, CSF and urine. Other sample types may be tested, and are reported alongside a caveat to state that the assay is not UKAS accredited for testing such sample types. Turnaround times are stated in the user manual (<http://www.micropathology.com/customer-downloads-handbook.php>) with results usually available in practice much sooner than the given timeframe. Where there is a delay, we are usually confirming a result and addressing clinical data given with the specimen.

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<sup>4</sup> Yaakob, Y., Rodrigues, K. and John, D. 2015. Leptospirosis: recent incidents and available diagnostics - a review. *The Medical journal of Malaysia*, vol. 70, no. 6, pp. 351-355.