Changi General Hospital
SingHealth

Tel: (65) 6788 8833
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Changi General Hospital
2 Simei Street 3
Singapore 529889
www.cgh.com.sg
Reg No 198904226R

Test Name	Leptospira species PCR [In-house Test]
Specimen Type	4 x Blood samples in EDTA tubes or 30 ml of urine sample or 1 ml of CSF
Special Instructions For Laboratory	-
Specimen Storage And Transport	Store specimen at 4°C. Transport to laboratory as soon as possible.
Specimen Minimum Volume	-
Test Method	Real-time PCR, lab developed test (non-FDA approved). This assay involves real-time polymerase chain reaction (PCR) targeted the lip32 gene of pathogenic Leptospira species, and the rrs (16S ribosomal RNA) gene of pathogenic and intermediate Leptospira species. The sequences used in the PCR test are based on two published studies. Leptospira species in the saprophytic group will not be detected by this assay. ***Reference*** Ahmed AA, Goris MG, Meijer MC. Development of lipL32 real-time PCR combined with an internal and extraction control for pathogenic *Leptospira* detection. Plos one. 2020 Nov 2;15(11):e0241584. Pérez LJ, Lanka S, DeShambo VJ, Fredrickson RL, Maddox CW. A validated multiplex real-time PCR assay for the diagnosis of infectious *Leptospira* spp.: a novel assay for the detection and differentiation of strains from both pathogenic groups I and II. Frontiers in microbiology. 2020 Mar 20;11:457.
Expected Result	Detected, Not detected, Inhibitory
Reference Ranges	Refer to Lab report
Turn Around Time	1-3 days
Days Of Testing	Monday - Saturday
Hospital	CGH
Laboratory	Microbiology Lab
Discipline	Microbiology

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<table>
<tr><td>Contact Details</td><td>4935 / 4936</td></tr>
<tr><td>CPOE Order Name Synonyms</td><td>-</td></tr>
<tr><td>Clinical Information</td><td>Patients with suspected infections caused by pathogenic Leptospira species.

In the acute phase of illness, leptospires are present in the blood (septicaemia) for approximately the first 4–10 days of illness. Leptospires may be shed intermittently in the urine after approximately the first week of illness onset.

Recommended diagnostic specimens based on collection timing
<table>
<tr><th></th><th>DAYS POST-ONSET</th><th>TEST TYPE</th><th>SAMPLE</th></tr>
<tr><td rowspan="3">Acute illness</td><td rowspan="3">first 10 days</td><td>PCR, blood</td><td>4 tubes EDTA (lavender top)</td></tr>
<tr><td colspan="2">AND</td></tr>
<tr><td>PCR, urine</td><td>urine (30 ml, sterile container)</td></tr>
<tr><td rowspan="3">Convalescent illness</td><td rowspan="3">after 10 days</td><td>PCR, urine</td><td>urine (30 ml sterile container)</td></tr>
<tr><td colspan="2">AND</td></tr>
<tr><td>Serology, IgM</td><td>3 ml plain blood (gold top)</td></tr>
</table>

Serology (performed in SGH)

Antibodies for leptospirosis develop between 3-10 days after symptom onset, thus any serologic test must be interpreted accordingly – negative serologic test results from samples collected in the first week of illness do not rule out disease, and serologic testing should be repeated on a convalescent sample collected 7-14 days after the first.</td></tr>
<tr><td>Link Out For Additional Information</td><td>-</td></tr>
<tr><td>Remarks</td><td>Performance Characteristics

1. EFFICIENCY AND LINEAR RANGE
The dual-target assay covers a linear range from 24 copies to 2.4 x 10^8 copies per PCR reaction for the lipL32 gene target, and from 1.93 x 10^2 copies to 1.93 x 10^8 copies per reaction for the rrs (16SrRNA) gene target. The PCR efficiency of the lipL32 gene target is 96%, and the PCR efficiency of the rrs (16SrRNA) gene target is 95%.

2. LIMIT OF DETECTION
20 replicates of two plasmids harbouring the gene target lipL32 and the gene target rrs were subjected to the PCR assay. The 95% LOD of this assay, i.e. 19/20 replicates detected, is 23 copies per PCR reaction for the lipL32 gene target, and 97 copies per PCR reaction for the rrs gene target.

3. SENSITIVITY AND SPECIFICITY
The in-vitro (analytical) sensitivity of the assay was initially established using two cultured strains of Leptospira interrogans and Leptospira borgpetersenii provided by the National Environmental Agency. Further in-vitro sensitivity was evaluated against an External Quality Assurance program conducted by the National Reference Laboratory, Australia.

In-vitro (analytical) specificity was evaluated against 3 bacterial isolates and 2 Candida sp.</td></tr>
</table>

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isolates, while clinical specificity was evaluated against 105 blood, urine and CSF specimens.

Because of the rarity of clinical leptospirosis in Singapore, no claims are made for clinical sensitivity.

Analytical sensitivity: 100%	Clinical sensitivity: no data
Analytical specificity: 100%	Clinical specificity: 100%

Based on the original publications from which the assay was derived, the clinical performance of the various gene targets is listed below.

GENE TARGET	**DIAGNOSTIC SENSITIVITY**	**DIAGNOSTIC SPECIFICITY**	**LEPTOSPIRAL TARGETS**
lip32	93.0% (CI 83.6%–97.4%)	98.3% (CI 94.8%–99.6%)	Pathogenic serovars
rrs	59.6% (CI 49.6%−68.8%)	96.2% (CI 94.4% − 97.6%)	Pathogenic AND intermediate serovars