Assessment of Real Time Polymerase Chain Reaction (PCR) Assay for the Early Diagnosis of Leptospirosis in Humans

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Leptospirosis is the most widespread zoonotic disease worldwide having a great impact on health issues in developing countries. It is caused by a pathogenic spirochete of the genus Leptospira where humans become infected through contact with the urine of infected animals. It is often exceptionally under-recognized as the clinical manifestation mimics variety of similar disease conditions that occur in the same environmental and climatologic conditions which accentuate the importance of laboratory diagnosis of leptospirosis. At present, no hospital based facilities are available for acute confirmation of the disease. The existing practice is retrospective confirmation with serological diagnosis. Therefore, the establishment of acute phase diagnosis will help in monitoring the disease, determining when hospital admission is required and reduce case fatalities. The objective of this study was to establish and evaluate a molecular-based assay to provide laboratory confirmation of leptospirosis at the acute phase of the infection (1-5 days of fever). Patients fulfilling clinical criteria stipulated by the accepted case definition were selected for the study and patients who failed to show evidence of sero conversion were considered as true negatives. A real time Polymerase Chain Reaction (PCR) assay with targeting a 203 bp fragment in the secY gene which is conserved among pathogenic serovars of Leptospira was established using a reference DNA sample (L.interrogans serovar Icterohaemorrhagiae strain RGA). Analytical sensitivity and the analytical specificity of the assay were calculated. The accuracy of the real time PCR was determined by a panel of acute blood samples collected from laboratory confirmed leptospirosis patients (n=35) and non-leptospirosis (n=44) patients based on Microscopic Agglutination Test (MAT) and/or IgM immunochromatography. Patients who failed to give positive test results either with MAT or IgM immunochromatography were considered as true negatives. Analytical sensitivity was approximately 314 genome equivalents per reaction and analytical specificity showed no amplification of Leptospira saprophytic sp. and other micro-organisms. The assay could effectively detect Leptospira DNA from clinically diagnosed leptospirosis suspected patients with 60.0% (21/35) diagnostic sensitivity and 77.27% (34/44) diagnostic specificity. This may be attributed to some samples failing laboratory confirmation despite their collection based on clinical suspicion. Therefore, real time PCR established can be used for rapid and definitive diagnosis of leptospirosis during the acute phase of infection.

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