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PAPER

Application of a Real Time Polymerase Chain Reaction (PCR) for Detection of Pathogenic *Leptospira* in Clinical Samples

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Leptospirosis, is a zoonotic disease with worldwide distribution, caused by pathogenic species of the genus *Leptospira*. It has the greatest impact on health in developing countries where it is often grossly under-recognized. Clinical features are similar to a range of other infectious diseases that occur in the same environmental and climatologic conditions. Therefore, laboratory confirmation is essential for proper management of leptospirosis patients. Molecular assays offer definitive laboratory confirmation of leptospirosis at the early phase of infection (1-5 days of fever) within a few hours.

The objective of this study was to establish and evaluate potential use of a real time- PCR assay for early, definitive laboratory confirmation of leptospirosis patients.

A SYBR green-based real time PCR assay targeting a 203 bp fragment on the secY gene which is conserved among pathogenic serovars of *Leptospira* was established using a reference DNA sample (*Leptospira interrogans* strain RGA). Analytical specificity of the assay was tested with the DNA from pathogenic and non-pathogenic *Leptospira* spp. and five other micro organisms. Analytical sensitivity of the assay was tested using serial dilutions of the reference sample. A panel of acute blood samples (n=150) collected during early phase of infection (1-5 days of fever) from leptospirosis suspected patients was used for evaluation of real time PCR vs qualitative PCR.

The results show, real time PCR assay with high analytical specificity (100%) was established and the assay shows 100 times higher sensitivity over qualitative PCR assay (1.3 pg/ml). Real time PCR and qualitative PCR could diagnose current leptospirosis infection in 37.3% (56/150) and 19.3% (29/150) suspected patients respectively. These results indicate high sensitivity of real time PCR over qualitative PCR for diagnosis of leptospirosis patients.

In conclusion, this study shows that real time PCR has the potential to facilitate rapid and sensitive diagnosis of acute leptospirosis during early phase of infection.

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