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A comparative study of molecular-based diagnostic assays for early definitive diagnosis of human leptospirosis

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Early diagnosis of leptospirosis infection helps in monitoring the disease, determining when hospital admission is necessary and reducing case fatalities. The objective of this study was to carry out a comparison of three molecular-based assays for early definitive diagnosis of leptospirosis. Three molecular-based assays; qualitative Polymerase Chain Reaction (PCR), quantitative real time PCR and Loop Mediated Isothermal Amplification (LAMP) assay were established and analytical sensitivity and specificity were checked. Paired blood samples were collected from leptospirosis suspected patients. Five millilitres of an acute blood sample were drawn from each patient at the early symptomatic phase (1-5 days of fever). A convalescent blood sample was collected after 7-14 days of the collection of acute blood sample. Laboratory conformation of each patient was performed by Microscopic Agglutination Test (MAT) and IgM immunochromatography on convalescent blood samples. Diagnostic capability of each molecular-based assay was tested using a panel of acute blood samples (n = 60) collected from leptospirosis confirmed patients. The same template DNA of each sample prepared using the same method was used for each molecular-based assay. Ethical permission for this study was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Kelaniya. Analytical specificity performed using the same reference samples showed that each molecular-based assay was highly specific only for leptospirosis. Analytical sensitivity of qualitative PCR, quantitative real time PCR and LAMP assay were 49 pg/ml, 1.3 pg/ml and 49 fg/ml respectively. As early laboratory diagnostic methods qualitative PCR, quantitative real time PCR and LAMP assay diagnosed only 25% (15/60), 65% (39/60) and 95% (57/60) laboratory confirmed leptospirosis patients. Quantitative real time PCR and LAMP could diagnose a large number of patients. These molecular-based diagnostic assays using a single serum specimen offers distinct advantages over other diagnostic techniques for early and definitive laboratory diagnosis of leptospirosis infection when serological methods are of little value.

Keywords: leptospirosis, early, diagnosis, molecular-based assays

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