Designing a Positive Control for Molecular Diagnosis of Pathogenic Leptospires by PCR Based on LigB Gene

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Background & Objectives: Leptospirosis, a zoonosis caused by bacteria of the genus Leptospira, is an important emerging infectious and the most prevalent zoonotic diseases in theworld. LigB is an immunogenic protein found in pathogenic Leptospira spp. The protein expressed by LigB gene may be used in diagnostic Methods and also can be a good candidate for recombinant vaccine against leptospirosis. The aim of this study was designing a positive control for molecular diagnosis of pathogen Leptospires by PCR based on ligB gene.

Methods: In this study five pathogenic serovars and one saprophyte specie of Leptospirawere used to inoculate into the selective culture medium and extraction of the genomic DNA by standard Phenol-Chlorophorm Methods. The specific primers for proliferation of ligB gene were designed. The PCR product of Leptospira interrogans, Serovar Serjoe hardjo was purified using kit. Thereafter, eluted DNA was ligated in pJET1.2/ blunt vector. The cells were exposed to heat shock and transformed in competent *E.coli* Top10 cells.The recombinant plasmid were extracted using kit.

Results: PCR amplification of the ligB gene using the designed primers resulted in a 1041 bp ligB gene product. The PCR based on ligB gene detected all pathogenic reference serovars of Leptospira spp. tested. No PCR products were amplified from the non-pathogenic L.biflexa. The amplified gene was cloned in pJET1.2/ blunt vector and transformed into E. coli (Top10) cells. The confirmation of the recombinants was made by picking the white colonies and carrying out colony PCR amplification of the gene. Positive colonies plasmid vector was isolated from cells by kit. PCR test was carried out with positive control and PCR products were observed on gel electrophoresis.

Conclusion: Due to slow growth of leptospira, certain maintaining conditions and lack of access to all reference strains, using a fast and accurate molecular tests like PCR with a positive control to confirm its accuracy is essential. Therefore, the clonedligB gene in this study has been prepared in Leptospira Reference Laboratory,can be used as a positive control in all laboratories using the PCR test.

Keywords: Leptospirosis; PCR; LigB