Development of an ELISA assay for quality control of Lig proteins

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Leptospirosis is a spirochaetal zoonotic disease that has been recognized as an important emerging infectious disease in last 10 years. To solve this problem, vaccines have being developed as a potentially attractive intervention strategy against this disease, given the current lack of effective control measures. *Leptospira* immunoglobulin-like (Lig) proteins are a family of surface-exposed molecules in pathogenic *Leptospira* and therefore may serve as targets for protective immune responses. The Lig genes have an identical region called LigBrep and a non identical region called LigANI and LigBNI. The gene correspondent to LigANI protein was cloned in pET-100-D/TOPO vector, the recombinants were selected and transformed in expression host *E.coli* BL21 DE3 (star). After that, the expression and solubility of the clones were analyzed using different temperatures and buffers. The purification was performed in HPLC, using affinity chromatography (IMAC) as major method. After purification the proteins were stored and the integrity, solubility and antigenicity were analyzed by SDS-PAGE and *immunoblotting*. These techniques were adopted as quality control procedures and are evaluated rigorously by us, once the stability is the major problem of recombinant proteins. Besides, it is necessarily to assure the quality of the antigen used to formulate vaccines and reagents for diagnostic kit, so we developed an ELISA test using pool of serum from pacients with of leptospirosis. The data showed the potential of the methods to help us to aggregate more information about the quality control of the proteins produced in our laboratory.

Key words: recombinant proteins, ELISA, leptospirosis