

IVD_25 - Development and evaluation of an ELISA using a combination of recombinant proteins to diagnose Leptospirosis in humans

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Introduction: Leptospirosis is a worldwide spread zoonotic disease caused by the bacteria *Leptospira spp.* It can affect both humans and animals, most often by indirect contact with an environment contaminated with the urine shed by infected mammalian reservoirs. It usually occurs in tropical and subtropical areas of the world due to its frequency of rain. The common symptoms of this infection fail to discriminate this disease from other infectious diseases like dengue fever, malaria, and others. For this reason, early diagnosis is of extreme importance. Currently, the reference immunological test is the microscopic agglutination test. Still, it has some limitations such as false negative results in the early course of infection, and once it is difficult to perform, its use is restricted to reference laboratories.

Objectives: Therefore, to improve the national public health system, as well as diagnose the disease as quickly as possible to prevent fatal resolutions, the objective of this study was to standardize an enzyme-linked immunosorbent assay (ELISA) using recombinant proteins for establishing a new IgM-ELISA.

Methodology: Three *Leptospira spp.*'s recombinant proteins (L1, L2, and L3) that were better described in the literature were tested in an indirect ELISA platform. In the first analyses, the proteins were tested separately using anti-IgM conjugate. After establishing the best concentration for each protein, it was observed that the proteins L1 and L3 displayed better results. The protein L2 was excluded from.

Results: To improve the results, the proteins L1 and L3 were combined, and the concentrations were tested once again. After the best concentration was established, a panel with 189 sera was tested. Sensitivity and specificity were calculated with MedCalc Software and the agreement was calculated using the kappa index. As preliminary results, the sensitivity obtained was 91% (CI 78%-97%) and the specificity was 93% (CI 87%-96%). The agreement obtained by the kappa analysis was considered substantial (0.8).

Conclusion: Even though these preliminary results indicate the potential applicability of the test, more studies will be conducted aiming to perfect it.

Keywords: Leptospira; Recombinant protein; Diagnosis