

## SUMMARY OF THESIS\*

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### CONTRIBUTION TO THE IMMUNODIAGNOSIS OF HUMAN LEPTOSPIROSIS: EMPHASIS TO THE USE OF MONOCLONAL ANTIBODIES

The best serological test for leptospirosis laboratory diagnosis remains the microscopic agglutination test (MAT). Because of the complexity of MAT, we developed some rapid screening tests for leptospiral antibodies detection in the acute phase of infection. In the decade of 80, a passive hemagglutination test employing polysaccharide fractions of leptospire was considered appropriate for early diagnosis, but its antigen preparation included "common antigens" recognized by antibodies from 4% of healthy individuals. A new ELISA (enzyme-linked immunosorbent assay) employing proteinase K resistant immunodominant antigens was developed and its potential diagnosis evaluated. This technique, the PK-ELISA, presented 89.9% sensitivity and 97.4% specificity, and satisfied the requirements needed for serological screening tests of human leptospirosis. However, some of the reagents used in its antigen preparation are imported and very unstable. So, it was proposed, in a "Cooperative Research Accordance" between Instituto Adolfo Lutz and Laboratório Fleury, to try new approaches with monoclonal antibodies. Two hybridomas secreting specific monoclonal antibodies (MAb) were selected: one, against an epitope detected in 16 of 23 members of the genus *Leptospira* (clone A12P4) and the other, specific to the icterohaemorrhagiae serogroup (clone H7P1). The MAb A12P4, a G2 (IgG2B) immunoglobulin, reacted with an epitope present in the 16-18 kDa components of icterohaemorrhagiae serogroup and with the 75-84 kDa components of serovars copenhageni and canicola, after whole-cell lysates of the leptospire were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The MAb

H7P1, which is an IgG, reacted with an epitope common to several fractions of molecular weight above 21 kDa of strain RGA and with the 21-22 kDa and the 75-82 kDa components of strain M-20. Both monoclonal antibodies were employed in enzyme immunoassays for detecting specific antibodies in serum samples serially collected from 52 patients with leptospirosis, and from the control group, which consisted of sera from 57 patients with other diseases included in the differential diagnosis, and from 68 healthy individuals. These tests, however, were not satisfactory. A new ELISA was developed in the present study employing an antigen suspension "AgMc", purified by affinity chromatography with CNBr-activated Sepharose 4B coupled to the monoclonal antibodies described above. The results obtained with this test were compared to the MAT and to the classical IgM ELISA (ELISA c). The new method, "AgMc ELISA", presented serological indices, relatively to reference test MAT, of 80.70% and 83.33% of sensitivity and specificity, respectively; positive and negative predictive values of 69.70% and 90.10%, respectively, and general agreement index of 82.49%. So, this test was not considered a promising approach to rapid diagnosis of human leptospirosis. Moreover, the proportion of patients diagnosed as having leptospirosis by the "AgMc ELISA" and the MAT differ significantly. The possible explanations for the results obtained are discussed.

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