

RESEARCH NOTE

ELISA for the Detection of Human Leptospirosis

Ana Margarita Obregon Fuentes,
Anselmo Otero Gonzalez,
Jose Laferte Serrano

Instituto de Medicina Tropical "Pedro Kouri",
Laboratorio Nacional de Referencia de Leptospira,
Apdo. 601, Marianao 13, Ciudad de la Habana, Cuba

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The leptospirosis is an acute febrile zoonotic disease, widespread in different areas in Cuba. People become infected when they get in contact with leptospire excreted by the reservoir host and in certain occupations (eg: agricultural workers) where the risk of infection is high. For this reason human leptospirosis continues to be an important public health problem.

There are some reports in the literature describing the use of Enzyme Linked Immunosorbent Assay (ELISA) for detecting antileptospiral antibodies in human serum (AJ Champman 1988 *J Med Microb* 25: 269-278). Our laboratory has frequently received requests for information about the value of ELISA as a diagnostic test for leptospirosis.

In this study we used the Biflexa Microscopic Agglutination Test and the Passive Hemagglutination Test (as were described in the Guidelines for the Control of Leptospirosis) as reference for the confirmation of clinical and epidemiological diagnosis and also for the comparison between our results with ELISA (S Faine 1982 WHO Publications No. 67 Geneva).

Our ELISA was performed as described by WJ Terpstra et al. (1980 *Zbl Bakt Mikrob Hyg I Abt Orig A* 247: 400-405).

Antigen was prepared from a well-grown culture of *Leptospira biflexa* serovar *patoc* strain Patoc I.

The standardization assay was set up using positive (from patients with confirmed leptospirosis) and negative (blood bank) samples.

All sera were serologically confirmed by the reference test (referred before) as positive and negative.

An anti human (whole molecule) peroxidase conjugate was used to reveal the reaction. The cut off value was determined from a frequency histogram (0.3) using the blood samples and sera of patients with leptospirosis. A standard curve was constructed using the log of optical density and the end point titer of selected sera in order to estimate the titer of each sample at a single dilution (1:160).

We applied the ELISA technique to measure levels of human antibodies from 58 paired sera of patients with leptospirosis, 51 were positive (88%).

We studied a panel of sera from 200 healthy blood donors and 20 false positive reactions were found. In the control group we also included 14 patients with meningitis, 2 patients with respiratory disease, 34 with positive hepatitis B serology, 8 with a positive syphilis serology, 20 with toxoplasmosis, 5 with rubella and 5 with measles. The sera of patients having other infectious diseases were negative by ELISA.

The ELISA has the advantage that only one heat-extracted antigen is used which is sufficiently broadly reactive to detect antibodies induced by different serovars, besides it is able to study a large number of sera (from patients) and ELISA is an automatized system bringing objectively results, not being so expensive one time it was standardized (WJ Terpstra et al. 1987 *J Med East African* 64: 49-53).

A clear difference was observed (by ELISA) between the titres of leptospirosis patients and those of a control group. With the criterium (end point titer) 20 false positive were noted among a control group of 200 donors samples.

The coincidence between Passive Hemagglutination Test and ELISA was 88% and their discrepancy 12%; Microagglutination Test and ELISA coincided in 65% and discreped in 35%.

The sensitivity of ELISA was 88%, the specificity 90.5%. ELISA test may be useful as initial screening test for the rapid diagnosis (of individual cases) as well as for screening large numbers of sera (usual as in epidemiological surveys) taking into account the advantages of this method respecting with other serological tests used for diagnosis and epidemiological surveys.