SERODIAGNOSIS OF HUMAN LEPTOSPIROSIS BY IMMUNODOT ASSAY USING TRITON X-114 EXTRACTION LIPOPROTEIN ANTIGENS FROM OUTER MEMBRANE OF ENDEMIC LEPTOSPIRAL SEROVAR

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Abstract

Leptospirosis is a zoonotic disease of global importance. Early diagnosis is important in the treatment of leptospirosis. In this study, immunodot assay was developed for the detection of specific IgM antibodies using partially purified leptospiral antigen isolated from the outer membrane of the organism by using Triton X-114 extraction method. IgM dot-ELISA assay for serodiagnosis of acute leptospirosis was evaluated and compared with the standard microscopic agglutination test (MAT). Serovar Bratislava was chosen for preparation of antigen due to its being the most dominant serovar of Leptospira associated with disease in Thailand reported in recent studies. The outer membrane protein of the organism was extracted by the nonionic detergents Triton X-114, and then fractionated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The Triton X-114 detergent phase has been shown to contain outer membrane components, including several lipoproteins; i.e., LipL32, LipL36, LipL41. The optimal condition for IgM dot-ELISA testing was determined by the checkerboard titrations against known positive and negative standard sera. The IgM dot-ELISA performed with 370 serum samples at 1:40 dilution demonstrated the sensitivity, specificity, and efficiency of 97.78, 95.00 and 95.68% respectively. The IgM dot-ELISA assay showed the results with statistically significant differences from MAT (p<0.05). The stability test for the test strip was performed after storage at 4 °C and -20 °C at different times and the results showed good performance of the test strip at both storage temperatures for up to one year. The use of Triton X-114 method for extraction of lipoprotein from the outer membrane of the organism was found to be a simple and inexpensive method. When used this partially purified antigenic preparation in the IgM dot-ELISA, the assay was easy to perform with a visual reading of the results that did not require special equipment. Future studies to improve the stability of the test strip and assay reagents at room temperature are needed and more evaluation of the assay use in the field for screening of human leptospirosis should be performed.