Thesis Title Detection of Legionella Infection by using Broad-Spectrum Enzyme-Linked Immunosorbent Assay

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Abstract

Legionella infection causes two distinct clinical syndromes , a fulminating pneumonic illness known as Legionnaires' disease and an acute self-limited febrile non-pneumonia termed as Pontiac fever. The clinical symptoms of Legionnaires' disease cannot be differentiated from pneumonia caused by other organisms. Since legionellae resist to the antibiotics commonly used in most pneumonic patient,an early diagnosis of Legionnaires' disease is required in order to apply suitable therapy. The detection of Legionella antigen in urine specimen have been reported as an effective tool for diagnosis of Legionnaires' disease in the early stage of the illness. In this study , broad-spectrum double sandwich enzyme-linked immunosorbent assay have been established for the detection of Legionella soluble antigen in clinical specimens and water samples. Rabbit anti-L. pneumophila serogroup 1(Knoxville) and serogroup3 (Bloomington-2) IgG have been raised and used as the capture antibodies and for preparing antibodies alkaline phosphatase conjugats. The established ELISA using L.pneumophila

serogroup 1 IgG could detect homologous antigen prepared from culture extract at a concentration of $\geq$ 0.1 μg/ml of protein which dry weight was 68 ng/ml and could also detect antigens of other serogroups including that of serogroup 2, 3 , 4 and 5 at concentrations of $\geq$2.5 μg/ml protein, of serogroup 6 at concentrations of $\geq$ 5.0 μg/ml protein. The serogroup 3 ELISA could detect homologous antigen prepared from culture extract as little as 0.05 μg/ml of protein which dry weight was 23 ng/ml and also could detect antigens of serogroup 6 at $\geq$2.5 μg/ml of protein, of serogroup 4 and 5 at $\geq$ 5.0 μg/ml of protein and of serogroup 1 at $\geq$ 7.5 μg/ml of protein but could not detect serogroup 2 antigen at the protein concentration less than 20 μg/ml. The established serogroup 1 and serogroup 3 ELISAs could detect homologous sonicated or heated L. pneumophila cell suspensions at the concentration $>$ $3x10^5$ and $3x10^6$ cells/ml, respectively.

Clinical specimens obtained from a patient with serological diagnosis proven having Legionnaires' disease caused by L. pneumophila serogroup 3 were detected for Legionella antigen by using the ELISAs. The specimens included 3 sera and 1 urine samples but all gave negative results by both ELISAs. However, the sensitivity of the tests in detection of Legionella antigen in clinical samples cannot be evaluated as the samples from only 1 patient with Legionnaires'disease was available in this study. Fifty-five water samples from cooling towers and environmental sources, containing $<$10 CFU /100 ml to $3.3X10^4$ CFU/100ml of Legionella spp, gave negative results for the detection of Legionella antigen by using the ELISAs. The results indicated that the tests were not sensitive enough in detection of small amount of antigen in water samples. The specificity of the tests were found to be 100 percent since 205 serum samples and 94 urine samples obtained from patients with non-Legionella pneumonia , 109 urine samples obtained from patients with urinary tract infections and sera and urine obtained from 30 normal individuals , did not give positive

results by them.

In comparison of the established ELISAs to the passive staphylococcal agglutination tests , the homologous antigen detection ability of the ELISAs were higher than that of the passive staphylococcal agglutination which could detect homologous antigen at 0.5 μg/ml of protein except serogroup 3 antibody coated staphylococcal cells which could detect the antigen at 0.1 μg/ml of protein. Moreover , the ELISAs could detect heterologous antigens while the passive staphylococcal agglutination tests could not, except the test which used serogroup 6 antibody sensitized cells could detect soluble antigen of serogroup 3 at a concentration of 0.5 μg/ml. The specificity of both the ELISAs and the passive staphylococcal agglutination tests were as high as 100 percent but the sensitivity of them could not be evaluated since the lack of Legionnaires' disease patients during this study.