

## Scrub Typhus Rapid Diagnostic Test Kit Using dot-ELISA

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### Abstract

Scrub Typhus (ST) disease is a febrile illness caused by gram-negative intracellular bacteria, *Orientia tsutsugamushi*. The disease is endemic to the hospitals are always be managed without the specific diagnosis of ST infection at present are Weil-Felix test (WF), indirect immunoperoxidase test (IIP) and indirect immunofluorescent antibody test (IFA). The WF test, bases on the agglutination of OXK strain *Proteus mirabilis*, is the simplest procedure which is neither specific nor sensitive, but has been widely used to diagnose ST infection. The IFA test and IIP test, eventhough highly sensitive and specific, give satisfactory results in the hands of experienced personnel and can be troublesome for inexperienced technicians because of the final evaluation by microscopy. The purpose of this study was to develop a sensitive, specific and simple method for serodiagnosis of ST. The dot-enzyme linked immunosorbent assay (dot-ELISA) was successfully developed for detection of antibodies to scrub typhus in human sera because of the lack of a simple and reliable serodiagnostic technique which could be used in any laboratories. Scrub typhus antigens, the sonicated cultures of Karp, Gilliam, and Kato strains of *Orientia tsutsugamushi* in irradiated mouse L929 cells, were applied onto nitrocellulose discs in 96 well microtiter plates. The antigen nitrocellulose discs were incubated with skim milk, tested sera, anti-human IgM and IgG peroxidase conjugates and 4-chloro-1-naphthol, respectively. Positive reactions, the presence of antibodies of scrub typhus, resulted as purple-blue dots on nitrocellulose discs which were easily read by naked-eye. One thousand and forty-three sera from patients with acute fever from rural army hospitals and in Bangkok were collected to determine the specific IgM and IgG antibodies to scrub typhus by IFA test and dot-ELISA. It was found that 198 and 845 of 1,034 serum samples showed positive and negative by both IFA test and dot-ELISA, respectively. Only 9 sera gave different reactions between the two methods. Eight sera were considered positive by dot-ELISA but negative by IFA test. On the other hand, only one serum were considered negative by dot-ELISA but positive by IFA test. The dot-ELISA, with the 1:1,000 cut off titers for both IgM and IgG, was found to have sensitivity of 99.50%, specificity of 99.05%, efficiency of 99.23%, positive predictive value (PPV) of 96.12% and negative predictive value (NPV) of 99.88%, compared with the IFA test at the IgM titers  $\geq 1:400$  and the IgG titers  $\geq 1:400$ . In addition, dot-ELISA was not a time consumed method or a complicated antigen preparation especially the antigen nitrocellulose discs in 96 well microtiter plates could be kept for



one year at room temperature without the antigenic change. It was concluded that the dot-ELISA was a simple and outstanding technique for serodiagnosis of scrub typhus, was a more sensitive method than IFA test, was as suitable as IFA test for demonstrating the scrub typhus antibodies in human sera, had the distinct advantages over IFA test in that no particular expensive equipments were required and the results were easily determined by untrained personnel. The data suggested that dot-ELISA could be useful for serodiagnosis of scrub typhus and might be a practical substitute for IFA test.

