Thesis Title Detection of *Salmonella typhi* by enzyme immunoassay

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Date of Graduation May 31, 1988

ABSTRACT

Typhoid fever in human is caused by the bacterium *S. typhi*. Currently, the diagnosis is correlated to the detection of either bacteria by culture or antibodies in patients' sera by agglutination specific to the *S. typhi* (Vi, O_9, O_{12} antigens). The former method is relatively time-consuming whereas the latter is limited by low sensitivity. Therefore, the present study was attempted to detect *S. typhi* by using peroxidase-conjugated antibody.

Using 4.5×10^8 cells/ml, anti-Vi, anti-O_9 and anti-O_{12} were found to provide 50% agglutination at 1:320, 1:1600 and 1:400 respectively The enzyme immunoassay on microscopic slides could be accomplished within 3 hrs with 50% positive detection at 1:800, 1:1600 and 1:400 of the antisera. The sensitivity in detecting anti-Vi, anti-O_9 and anti-O_{12} was increased about 2 folds by using biotin-streptavidin and

further amplified (1:3200, 1:6400, 1:1600) with the application of biotin-avidin system.

Double antibody "sandwich" enzyme immunoassay was also explored. Immobilization of anti-O_9 (first antibody) on the polystyrene solid phase was established. However adsorption of formalin-fixed *S. typhi* on the immobilized anti-O_9 was not successful. Therefore, immune complex formation by peroxidase-conjugated anti-Vi (second antibody) in the subsequent reaction unobtainable. On the other hand, immobilization of *S. typhi* on titanous hydroxide followed by peroxidase-conjugated anti-Vi could detect the bacteria an about 900 cells. The detection was all positive for 28 *S. typhi* samples containing Vi antigen and totally negative for 20 samples of non-Vi antigen bacteria.