Evaluation of modified passive haemagglutination assay for Vi antibody estimation in Salmonella typhi infections

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Abstract
A simple passive haemagglutination assay (PHA) was developed to detect Vi antibodies, to improve the diagnosis of typhoid fever by small laboratories. The Vi capsular antigen of Salmonella typhi was extracted by alternate alcohol and acetone precipitation. Formalin fixed, sheep red blood cells treated with chromium chloride were sensitised with this Vi antigen and antibodies detected and measured by PHA. The test had a sensitivity of 83.3% among 30 cases of typhoid fever confirmed by culture. The specificity of the test was 94%, making it suitable for use in laboratories without facilities for IFAT or ELISA.

Typhoid fever continues to be a major problem in tropical developing countries. The facilities available for a confirmative microbiological diagnosis are often inadequate in such areas. Simple, economical, and reliable methods for diagnosis are therefore imperative. The conventional Widal test serves a useful but modest purpose. Many immunological tests such as indirect fluorescence antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) have been devised to detect Vi antibody in the serum of patients with typhoid fever and typhoid carriers. The passive haemagglutination assays (PHA) have been used for antibody detection in typhoid carriers. Those reported have relied on heat extracted Vi antigen or antigen prepared by ethanol-cetavlon precipitation. This report describes a modified method of Vi antigen preparation and evaluation of the PHA both in cases of typhoid fever and typhoid carriers.

Methods
Vi antigen was prepared from S typhi (ST) (NCTC 8382) grown on Mueller Hinton agar for 18 hours. The saline harvest of the organisms was heated at 100°C for one hour and centrifuged. The supernatant was treated with 2-5 volumes of absolute ethanol and proteins precipitated out. Centrifuged supernatant was treated with 3 volumes of acetone and the Vi polysaccharide was precipitated out and vacuum dried. Sheep red blood cells (SRBC) were fixed in formalin according to Csizmas’s method. Formalin (50 ml) was dialysed into 200 ml 12.5% (SRBC) in phosphate buffered saline (pH 7.4) (0.13 M) (PBS) for three hours and then formalin released into the SRBC suspension. This mixture was placed on a rotator overnight at room temperature. The coarse particles were removed and the SRBC suspension washed and stored at 4°C. A 4% suspension of SRBC was treated with chromium chloride (CrCl₃ : 6H₂O) at a concentration of 40 μg/ml for 15 minutes at 37°C with frequent mixing. After three washes in PBS, pH 7-4 (0-15 M), 2 ml of a 10% suspension of treated cells were mixed with 1 ml of Vi antigen solution (2 mg/ml) and incubated at 37°C for two hours with continuous mixing. The cells sensitised with Vi antigen were made into a 1% suspension in PBS, pH 7-4, containing 0-2% bovine serum albumin (BSA). A 1% suspension of unsensitised cells treated with chromium chloride was also prepared to serve as controls. All sera were absorbed with SRBC
Non typhoid fever (BC+W Widal test; Evaluation Confirmed typhoid Presumptive fever tube passive culture) 29 30 S 80 haemagglutination titre of non-typhoidal fever patients, was fixed Vi reagent (Vi serum were collected before and after vaccination against typhoid fever, for Vi antibody estimation in seven of 10 (70%) typhoid carriers. Of the five volunteers whose antibody titres were measured before and four weeks after vaccination against typhoid fever, all five showed a fourfold or greater rise in titre of antibodies to STH, two to STO, and none to ST Vi antigen. Among our study population the Widal test was positive in only 56% of blood culture positive patients; the PHA for Vi antibody estimation was positive in 83-3%. The PHA for Vi antibody detection therefore seems to be a good test for typhoid fever in small laboratories where culture facilities are not adequate or facilities for IFAT and ELISA are not available.

Results and discussion
Among the 100 healthy controls a geometric mean titre of 26-4 was observed in the tube agglutination test and 56-3 in the PHA Vi antibody test. The cutoff was 80 for tube agglutination and 160 for PHA. Of 30 confirmed typhoid fever patients, PHA was positive in 25 (83-3%) and tube agglutination in 10 (33-3%). Of 80 presumptive typhoid fever patients, 56 (70%) had PHA titres of 160 or more; only 36 (45-0%) had tube agglutination titres of 80 or more. Using positive blood cultures as the "gold standard" for diagnosing typhoid fever and healthy controls as disease negative, the PHA had a sensitivity of 83-3% and a specificity of 94% for diagnosing typhoid fever. The PHA did not give an increased false positivity among patients with non-typhoid febrile illness or connective tissue disorders. The results are shown in the table. The PHA test also detected Vi antibody in seven of 10 (70%) typhoid carriers.