

## Enzyme-Linked Immunosorbent Assay for Detection of Human Antibodies to *Salmonella typhi* Vi Antigen

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An enzyme-linked immunosorbent assay (ELISA) was developed to detect antibodies to *Salmonella typhi* Vi antigen in human serum, and the results were compared with those from a previously described hemagglutination assay (HA). The ELISA detected Vi antibodies at a titer of  $\geq 20$  in 40 (52%) of 77 sera from typhoid fever patients, whereas the HA gave titers of  $\geq 20$  in 35 (47%). Determination of titers of serum specimens from 170 persons without typhoid fever revealed Vi antibody titers of  $\geq 20$  in 4 (2.3%) by the ELISA and 3 (1.7%) by the HA. Unlike the sensitized erythrocytes used in the HA, the ELISA reagents have a shelf life of  $\geq 1$  year. The ELISA may be preferred by some laboratories, especially those already performing other ELISA tests.

Several investigators have used Vi antigen-coated erythrocytes to determine the titers of Vi antibodies in sera from suspected typhoid carriers (1, 2, 7). Nolan et al., using a more highly purified Vi antigen than was available to earlier investigators, were able to detect Vi antibodies in the sera of 22 (71%) of 31 current typhoid carriers (5) and to identify carriers in 7 (70%) of 10 outbreaks (6).

Using the same purified Vi antigen as Nolan et al., we developed an enzyme-linked immunosorbent assay (ELISA) for measurement of Vi antibodies and compared the ELISA with the hemagglutination assay (HA) in detecting Vi antibodies in the sera of persons with and without acute typhoid fever.

### MATERIALS AND METHODS

**Reagents.** Burro anti-*S. typhi* TY-2 plasma was obtained from John B. Robbins, Bureau of Biologics, Food and Drug Administration, Bethesda, Md. The plasma was clotted by the addition of bovine thrombin and centrifuged, and the serum was drawn off. Highly purified Vi antigen, prepared from *Citrobacter* 5396/38 by Cetavlon (Ayerst Laboratories, New York, N.Y.) precipitation as previously described (9), was provided by Kwei-Hay Wong, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Ga. Goat anti-human immunoglobulin G (IgG) was purchased from Antibodies, Inc. (Davis, Calif.) and coupled to alkaline phosphatase type VII (Sigma Chemical Co., St. Louis, Mo.) by the method of Voller et al. (8).

**Specimens.** Seventy-seven serum specimens were

obtained from patients with culture-confirmed typhoid fever in El Salvador. The 77 included 2 serum specimens collected at different times from 29 persons, but most were not appropriately timed to be true pairs. Serum samples were also obtained from 170 persons visiting health care centers in El Salvador for marriage licenses, foodhandler certificates, and other nonillness-associated reasons.

**ELISA procedure.** For the ELISA, optimal dilutions of all reagents were determined by checkerboard titration, and optimal times and temperatures for incubation were determined before assays were carried out.

Burro anti-*S. typhi* TY-2 was diluted 1:5,000 in 0.06 M carbonate buffer (pH 9.6) and mixed with Vi antigen at a final concentration of 1  $\mu$ g/ml. This mixture was then added (0.1 ml) to alternate rows of the inner wells of a polyvinyl microtiter plate (Dynatech Laboratories, Inc., Alexandria, Va.) with a U-shaped bottom. Burro serum without Vi antigen was added to the other rows of wells. The plates were incubated for 1 h at 37°C to allow the reaction to proceed and were then incubated overnight at 4°C to allow the proteins to attach to the microtiter plates. The plates were then washed three times with phosphate-buffered saline with 0.05% Tween 20 (PBS-Tween).

Serial twofold dilutions of patient sera were made in the microtiter plates with PBS-Tween with 1% fetal calf serum, beginning with a 1:20 dilution. Duplicate dilutions were made in both the Vi- and non-Vi-containing wells. When the diluting was completed, each well contained 0.1 ml of diluted serum. Serum-containing plates were then incubated for 1 h at 37°C and then washed three times in PBS-Tween.

Alkaline phosphatase-conjugated goat anti-human IgG was diluted 1:200 in PBS-Tween with 1% fetal calf serum, and 0.1 ml was added to each well. After incubation for 1 h at 37°C, the plates were washed three times with PBS-Tween. Enzyme substrate (Sigma 104 in diethanolamine buffer, pH 9.5, 1 mg/ml) was

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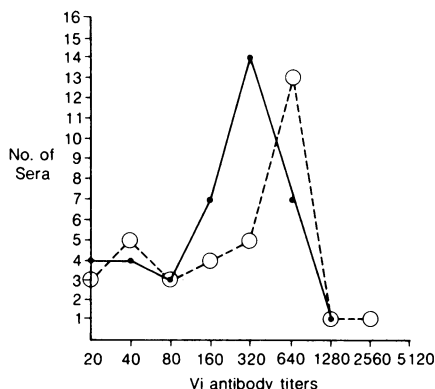


FIG. 1. Distribution of positive ( $\geq 20$ ) Vi antibody titers in sera from typhoid fever patients, as determined by the ELISA (—) and the HA (-----).

added, and the reaction was allowed to proceed for 30 min at room temperature and was then terminated by adding 0.025 ml of 3 N NaOH to each well.

Because we planned to use the ELISA in areas where spectrophotometers may not be available, results were read visually. Specimens were coded before receipt by the laboratory and were run blindly to avoid subjective interpretation as much as possible. Development of a yellow color in wells containing Vi antigen but not in wells without Vi antigen was considered positive. In most cases, the endpoint was reasonably sharp, rather than being a gradual fading of color intensity. The patient's titer was read as the reciprocal of the last dilution giving a positive reaction. When a serum gave some reaction in the lowest dilutions in the wells in which no Vi antigen was present, the titer was read as the reciprocal of the last dilution giving a positive reaction only if the reaction in the Vi-containing wells was at least 2 dilutions greater than that in the non-Vi-containing wells.

**HA procedure.** The HA procedure used at the Centers for Disease Control was performed as described previously by Nolan et al. (5).

## RESULTS

With the ELISA, we detected Vi antibodies at a titer of  $\geq 20$  in 40 (52%) of 77 serum specimens from typhoid fever patients, whereas with the HA, we detected titers of  $\geq 20$  in 35 (47%). Ten (13%) of 77 samples were positive (titer,  $\geq 20$ ) by the ELISA only, 5 (6.5%) by the HA only, and 30 (39%) by both assays. Of the 45 samples positive by either assay, 10 (22%) were positive by the ELISA only, 5 (11%) by the HA only, and 30 (67%) by both assays.

Among the 45 samples from typhoid fever patients positive by either assay, Vi antibody titers were higher by the ELISA in 15 (33%), higher by the HA in 17 (38%), and the same in 13 (29%). The difference in titers observed was less than 2 dilutions for 25 (56%) of 45 samples. Among the 20 with a difference of 2 or more dilutions, 12 (60%) were higher by the ELISA

than by the HA; the median interval between onset of fever and collection of serum in these cases was 16 days. The median interval between onset and collection for those higher by the HA was 29 days. Markedly elevated titers ( $\geq 160$ ) were observed in 30 (67%) of 45 positive samples by the ELISA and 23 (51%) of 45 by the HA (Fig. 1).

The distribution of the titers obtained by each method in sera from persons with typhoid fever is shown in Fig. 2. There was no significant difference between the titers obtained by the two methods ( $P = 0.132$ ;  $t$  test for paired data).

When the 40 specimens that were positive (titer,  $\geq 20$ ) by the ELISA were retested, 27 (67.5%) gave the same titer, 12 (30%) differed by 1 dilution, and 1 (2.5%) differed by 2 dilutions.

The precise time in the course of the illness when Vi antibodies detectable by these assays are produced could not be accurately determined from these sera because most were collected relatively late in the course of illness. Only one specimen was collected during the first 5 days after onset of fever, and 15 were collected during days 6 through 9. Antibody titers of  $\geq 20$  were detected in 8 (53%) of these 15 sera by the ELISA and 6 (40%) by the HA, proportions which differ little from the results for all serum specimens combined.

Determination of the titers of serum specimens from persons without typhoid fever revealed Vi antibody titers of  $\geq 20$  in 4 (2.3%) of 170 samples by the ELISA and 3 (1.7%) of 170 by the HA. One sample (0.5%) was positive by the ELISA only, none by the HA only, and 3 (1.7%) by both assays.

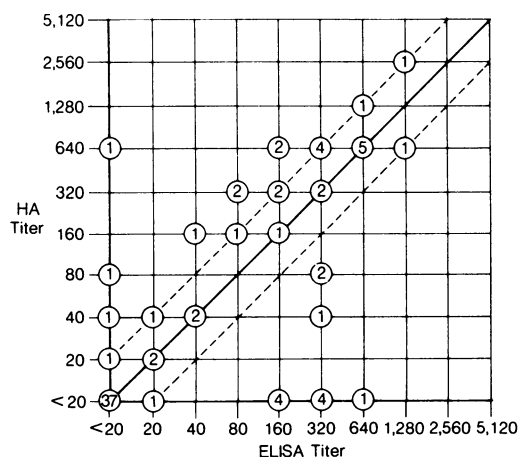


FIG. 2. Scattergram of Vi antibody titers obtained by the HA and the ELISA in 77 sera from persons with acute typhoid fever. Numbers inside data points represent number of sera with the specified titers. Numbers with dotted lines are those sera with titers in each test differing by no more than 1 twofold dilution.

## DISCUSSION

This paper describes an ELISA for the detection of Vi antibodies and compares the results obtained with this method to those obtained with hemagglutination, a previously described, proven method of detection (5). The results obtained by using the ELISA were very similar to those obtained with the HA. Most (67%) specimens that were positive (titer,  $\geq 20$ ) by either assay were positive by both assays. Five more specimens were positive by the ELISA only than were positive by the HA only. The specificities of the two assays appear to be quite similar, since only 1 of 170 sera from persons without typhoid fever was positive by one assay alone (ELISA titer, 40).

Hemagglutination tests in general should be more responsive to IgM than to IgG antibodies, whereas the ELISA uses a supposedly class-specific anti-IgG conjugate. Some differences in results might be expected for this reason alone, particularly very early or very late in infection when IgM or IgG antibodies, respectively, should predominate. Our data do not provide evidence supporting this hypothesis and actually show a tendency for ELISA titers to be higher early in infection. However, the titers and rates of positivity did not differ significantly.

The ELISA offers two advantages over the HA. When a difference in titers of more than 1 dilution was observed, the ELISA usually gave the higher titer, and high titers ( $\geq 160$ ) were also seen more often with the ELISA. Unlike the sensitized erythrocytes used in the HA, the ELISA reagents are stable for a year or more at 4°C. The labeled anti-human IgG may also be used in other ELISA systems, providing a further advantage to laboratories already performing other ELISA tests.

The advantages of the HA include greater ease of visual reading and theoretically better detection of early (IgM) response. Laboratories performing other HA tests may prefer the HA,

particularly if they have no experience in using the ELISA.

An evaluation of the possible uses of measurements of Vi antibodies is beyond the scope of this paper. Nolan et al. (5), Chitkara and Urquhart (4), Chau and Chan (3), and others have demonstrated the usefulness of Vi antibody titers in detecting typhoid carriers, but Vi serology has rarely been applied to diagnosis of acute typhoid fever. The ELISA has been shown to be as good as or better than the HA for detecting Vi antibodies and should be considered by those wishing to investigate this problem.

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