

Improvement of the Indirect Hemagglutination Assay for *Salmonella typhi* Vi Antibodies by Use of Glutaraldehyde-Fixed Erythrocytes

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Glutaraldehyde-fixed sheep erythrocytes sensitized with Vi antigen were shown to be usable for at least 1 year in the indirect hemagglutination assay for Vi antibodies. The use of fixed sensitized erythrocytes improves the practicality of the indirect hemagglutination assay by (i) eliminating the need to sensitize cells each time the test is performed; (ii) reducing waste of the purified Vi antigen; and (iii) reducing test-to-test variation.

An indirect hemagglutination assay (IHA) with sheep erythrocytes coated with Vi antigen has been used by several investigators to determine the titer of antibodies to Vi antigen in serum samples from suspected typhoid carriers (4-7). Nolan et al. were able to use IHA Vi serology to detect Vi antibodies in the sera of 22 (71%) of 31 current typhoid carriers (6) and to identify carriers in 7 (70%) of 10 typhoid outbreaks (7). In these investigations, fresh sheep erythrocytes were sensitized with Vi antigen each test day. Lanata et al. used glutaraldehyde-fixed cells but did not state whether cells were sensitized before each test or used over a period of time, thus eliminating the time-consuming need for sensitizing cells before each run (4). Their results confirmed the usefulness of the IHA with Vi antigen, but they did not report their laboratory methods, compare the results obtained with fresh versus fixed cells for the same specimens, or report on the stability of the fixed sensitized cells. This study, which presents such a report, had two purposes: (i) to compare the results obtained with fresh sensitized cells versus fixed sensitized cells and (ii) to determine the stability of the fixed sensitized cells over time.

Citrated sheep erythrocytes were fixed with glutaraldehyde by a modification of the method of Bing (2). Fresh cells were washed three times in 0.85% saline, and then 9 volumes of 1% glutaraldehyde (25% glutaraldehyde diluted in saline) were added to each volume of packed cells. The erythrocytes were then suspended by gentle stirring and left at room temperature for 2 h with occasional mixing. The cell suspension was then centrifuged, the supernatant was discarded, and the packed cells were suspended in 0.85% saline. Three washes in saline were followed by two washes in distilled water. Finally, a 10% suspension of fixed erythrocytes was prepared in saline containing 0.1% sodium azide as a preservative. The suspension was stored at 4°C until needed. Vi antigen was purified from *Citrobacter* sp. strain 5396/38 by Cetavlon precipitation as previously described (8).

Fresh and fixed sheep erythrocytes were both sensitized by the following procedure. After being washed three times in phosphate-buffered saline (PBS) (pH 7.2), cells suspensions were diluted to 1% (vol/vol) in PBS. An equal volume of PBS containing Vi antigen (10 µg/ml) was added, and the suspensions were mixed and incubated for 2 h in a 37°C water bath. After being washed three times in PBS, the sensitized cells were finally suspended at a concentration of 0.5% in PBS containing 0.06% bovine serum albumin. Fresh

cells were used immediately. Fixed cells were stored at 4°C for up to 1 year. Serum titers were determined as previously described (6).

Fresh and fixed cells were compared by testing paired serum specimens from 22 persons with culture-confirmed typhoid fever and single serum specimens from 18 persons suspected of being chronic carriers of *Salmonella typhi* for Vi antibodies by both methods. The stability of the fixed sensitized cells was determined by using one lot of cells to titrate 7 paired and 34 unpaired specimens as they were received over a period of 1 year at the Centers for Disease Control. *S. typhi* O and H antibodies were also assayed by standard methods (3), and Vi antibody titers were determined by an enzyme-linked immunosorbent assay (ELISA) (1) as well as by the IHA.

The titers obtained with this lot of fixed cells were somewhat higher overall than the titers obtained with fresh cells (11 of 62 were 2 dilutions higher, and 1 of 62 was 3 dilutions higher). Although a fresh-cell titer of ≥ 40 was established as the positive cutoff value in previous work (1), this value resulted in several apparent false-positives when applied to the fixed-cell assay. Raising the positive cutoff value for fixed cells to ≥ 80 yielded positive and negative results essentially identical to those of the fresh-cell assay (Table 1). Sera from 9 of 22 (40%) culture-positive persons were positive (titer, ≥ 80) with fixed cells, whereas sera from 8 of the 22 (36%) persons were positive (titer, ≥ 40) with fresh cells. ELISA serology identified the same nine positives as did the fixed-cell assay. When these same positive cutoff values were used, 2 of 18 (11%) suspected carriers had positive titers by both methods as well as by ELISA. None of the 18 possible carriers had positive Vi titers by either IHA method in the absence of other supporting evidence (positive O, H, or ELISA Vi titers). Although these cutoff values resulted in no false-positives among 16 apparently normal persons, only one lot of fixed sensitized cells was evaluated. As some variability in titer may occur with different lots of cells, each lot should be titrated with known positive and negative sera before positive cutoff values are assigned.

The 48 sera received as reference specimens at our laboratory were tested at irregular intervals over the 1-year period. Tests were performed on 10 separate occasions. Only 1 of 48 (2%) had a positive IHA Vi titer in the absence of other evidence of *S. typhi* infection (positive culture or

TABLE 1. Number of serum specimens with indicated titers in the IHA with fresh or fixed erythrocytes coated with Vi antigen

Erythrocytes	No. of serum specimens with indicated titer					
	≤20	40	80	160	320	>640
Fresh	48	5	2	3	3	1
Fixed	35	12	6	4	5	0

serologic test). Of the 48, 4 (8%) had positive Widal assay titers in the absence of a positive culture or Vi test. Four had positive IHA Vi, ELISA Vi, and Widal assay titers. None were positive in the ELISA only. Positive control-serum titers did not decline over the year and were within 2 dilutions of the initial value at every testing interval, indicating no significant change in titer.

As the titer of the positive control serum remained stable and as the number of apparent false-positives did not increase over the course of testing, the fixed sensitized cells were usable for at least 1 year. A significant saving of time is realized as compared with sensitizing cells whenever needed, especially in laboratories at which specimens are received at infrequent intervals. The use of the same lot of sensitized cells also enables a laboratory technician to more accurately compare results obtained over an extended period. Not having to prepare freshly sensitized cells also reduces waste, an important factor because purified Vi antigen is not commercially available. The use of fixed sensitized erythrocytes, which can be stored for up to 1

year, improves the practicality of the IHA for many laboratories.

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