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Indirect Hemagglutination Test for Human Antibody to Typhus and Spotted Fever Group Rickettsiae

AKIRA SHIRAI, JOHN W. DIETEL, AND JOSEPH V. OSTERMAN*

Department of Hazardous Microorganisms, Walter Reed Army Institute of Research, Washington, D.C. 20012

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An indirect hemagglutination (IHA) test is described that uses glutaraldehyde-stabilized erythrocytes treated with a rickettsial erythrocyte-sensitizing substance obtained from *Rickettsia typhi* or *Rickettsia rickettsii*. The serological reagent was stable for at least 3 months at room temperature and 6 months at 4 C. It exhibited group specificity and no group cross-reactivity. At a minimum dilution of 1:40, acute and early convalescent epidemic and murine typhus antisera showed 86% positive reactors, whereas similar spotted fever antisera had 74% positive reactors. In comparison with the indirect fluorescent antibody test, the IHA procedure gave lower titers but showed comparable detection of seroconversion with most paired sera. The IHA test demonstrated significantly higher titers than the complement fixation test and was more sensitive than either the complement fixation or Weil-Felix test in identifying seroconversion. No agglutination was observed when sensitized erythrocytes were tested with rodent sera known to contain rickettsial antibodies.

The indirect hemagglutination (IHA) technique has been used to study rickettsial infections by sensitizing erythrocytes with extracts of *Proteus* OX strain bacteria (9) or rickettsial organisms (1, 4, 5). The *Proteus* OX strains are easily grown in large quantities and provide a convenient source of erythrocyte-sensitizing substance (ESS), but certain rickettsial infections, such as Brill-Zinsser disease (6) and rickettsialpox (5), do not elicit the production of Weil-Felix (WF) agglutinins. Treatment of erythrocytes with rickettsial ESS broadens the scope of the test to include both these diseases, but erythrocyte fragility necessitates frequent sensitization of fresh cells and restricts use of the technique in field situations. This paper describes an IHA test for rickettsial antibodies that uses rickettsial ESS bound to glutaraldehyde-stabilized erythrocytes. The test retains the sensitivity and simplicity of the original technique, but broadens its application by removing the need for frequent sensitization and standardization of erythrocytes and by extending its practical use to field studies.

MATERIALS AND METHODS

Preparation of glutaraldehyde-treated (fixed) sheep erythrocytes. Fresh sheep erythrocytes in Alsevers solution (3) were washed and resuspended to 20% (vol/vol) in phosphate-buffered saline (PBS), pH 7.2. Equal volumes of 20% cells and 0.2% glutaraldehyde in PBS were mixed, incubated at 37 C for 15 min, washed five times in 0.85% normal saline, and suspended to a final concentration of 10% (vol/vol) in PBS containing 0.1% sodium azide (NaN_3). This 10% stock suspension was stored at 4 C.

Preparation of ESS. ESS was prepared by the method of Chang et al. (5). Yolk sac membranes, infected with either *Rickettsia typhi* or *Rickettsia rickettsii*, were blended in normal saline containing 0.1% NaN_3 to yield a 20% (wt/vol) suspension and centrifuged at 3,020 × *g* for 1 h at 4 C. The supernatant was discarded, the sediment was resuspended to the original volume in normal saline containing 0.1% NaN_3, and an equal volume of anhydrous ethyl ether was added. The mixture was shaken vigorously for 5 min and allowed to settle for 1 h, and the aqueous phase was removed and adjusted to 0.2 N NaOH. The alkaline suspension was heated at 100 C for 30 min, cooled, and dialyzed against three changes of PBS over 72 h. The ESS was stored undiluted at 4 C and diluted 1:5 in McIlvaine buffer solution (MBS) (13) before use in erythrocyte sensitization. Chemical quantitation was performed by the methods of Lowry et al. (12) and Dubois et al. (8).

Sensitization of glutaraldehyde-treated (fixed) sheep erythrocytes. An aliquot of 10% fixed erythrocytes was concentrated by centrifugation at 270 × *g* for 8 min, the supernatant was discarded, and the pellet was washed with MBS. The pellet was diluted 1:20 in ESS, and the mixture was incubated at 37 C for 1 h with frequent shaking. Sensitized cells were washed three times in MBS and resuspended in PBS containing 0.4% bovine serum albumin and 0.1% NaN_3 to make a final 0.5% (vol/vol) suspension, which was stored at 4 C. Control cells were prepared similarly except that the fixed erythrocytes were mixed with MBS instead of ESS.

Sera. Most of the human sera used in this study were obtained from clinically documented cases of

rickettsial disease and were further characterized and validated by complement fixation (CF) and indirect fluorescent antibody (IFA) tests. The following rickettsial diseases were represented: (i) murine typhus (*R. typhi*)—eight sera received from C. Sweet, Texas State Department of Health, Austin, Tex.; 23 sera received from Vietnam by the Rickettsial Disease Section, Walter Reed Army Institute of Research, Washington, D.C. (not clinically documented); (ii) epidemic typhus (*R. prowazekii*)—20 sera, received from J. J. Plorde, Department of Medicine, University of Washington, Seattle, Wash.; (iii) Brill-Zinsser disease (*R. prowazekii*)—three sera, received from E. Murray, Harvard School of Public Health, Boston, Mass.; (iv) Q fever (*Coxiella burnetii*)—16 sera, received from E. H. Lennette, California State Department of Health, Berkeley, Calif.; (v) scrub typhus (*R. tsutsugamushi*)—102 sera, received from D. M. Robinson, U.S. Army Medical Research Unit, Kuala Lumpur, Malaysia (100 sera not clinically documented); (vi) Rocky Mountain spotted fever (*R. rickettsii*)—15 sera received from C. Shepard, Leprosy and Rickettsia Branch, Center for Disease Control, Atlanta, Ga.; 19 sera received from F. Lambert, Commonwealth of Virginia Division of Consolidated Laboratory Services, Richmond, Va.; and eight sera received from C. Linnemann, Jr., Infectious Disease Division, University of Cincinnati Medical Center, Cincinnati, Ohio. In addition, 14 normal human sera were used for negative controls. All sera were stored at −40 C.

IHA test. The test was adapted to the microtiter technique (15) to conserve reagents. Serum samples, initially diluted 1:40, subsequently underwent serial twofold dilutions in the wells of U-type microtiter plates containing 0.025 ml of PBS-0.4% bovine serum albumin-0.1% NaN_3, followed by the addition of 0.025 ml of sensitized erythrocytes to each well. Nonsensitized erythrocytes were incubated with the lowest dilution of each serum used in a test, as a control for nonspecific agglutination. Plates were sealed with plastic tape, gently agitated, and maintained at room temperature (approximately 26 C). After 2 and 18 h of incubation, the plates were inspected for agglutination and results were recorded as 1+ (positive; smooth mat of cells covering bottom of cup, edges occasionally folded); +− (borderline; small mat of cells surrounded by compact circle of sedimented cells); or 0 (negative; button of cells forming small open circle or compact button of sedimented cells in center of well). The end point was defined as the maximum dilution exhibiting 1+ agglutination.

Confirmatory serological tests. The IFA test was performed as described by Bozeman and Elisberg (2), and the minimum titer accepted as significant was 1:40. The CF test was performed by the CF 52 method (11), modified for the microtiter technique, and the minimum titer accepted as significant was 1:8. WF tests were performed by the macroscopic agglutination technique (14), and the minimum titer accepted as significant was 1:160.

RESULTS

Antigen titration. Stabilized erythrocytes were sensitized with various dilutions of rickettsial ESS and tested against serial dilutions of positive control serum. A 1:5 dilution was found optimal for both maximum titer and sharpness of end point. This dilution of typhus ESS contained 59 μg of protein and 3.5 μg of carbohydrate per ml; spotted fever ESS contained 54 μg of protein and 6.5 μg of carbohydrate per ml.

Stability of components. The assembled IHA reagent, composed of stabilized, sensitized erythrocytes, was stored for 3 months at room temperature and 6 months at 4 C with no change in titer when periodically tested against positive control sera. Figure 1 includes a comparison of the agglutination pattern achieved with freshly sensitized cells and those stored as complete reagent for 3 months at room temperature. The effect of storage at 4 C on the assembled IHA reagent is shown in Table 1. The titers achieved with four control sera showed that the reagent was quite stable for 6 months, but continued storage for a total of 12 months resulted in fourfold decreases in antibody titers. The results shown in Table 2, which used a larger number of sera, also reflect two- to fourfold decreases in antibody titers after 12 months of storage of the complete reagent; however, it is seen that separate storage of individual components for the same period of time, with sensitization performed at time of testing, gave identical results to those achieved by using freshly prepared erythrocytes and ESS.

Specificity of reaction. Figure 1 shows a representative IHA test using antisera from patients with various rickettsial diseases. Only those antisera from Brill-Zinsser disease and epidemic or murine typhus infections agglutinated erythrocytes sensitized with ESS from *R. typhi*. The titer of the murine typhus antiserum in (A) and (H) is 1:10,240, the epidemic typhus antiserum in (B) is 1:640, and the Brill-Zinsser serum in (C) is 1:2,560. Figure 2 shows a similar IHA test using erythrocytes sensitized with ESS from *R. rickettsii*. Only the antisera of patients with Rocky Mountain spotted fever caused erythrocyte agglutination. The titer of the spotted fever serum in (A) is 1:10,240.

Comparison of specificity and sensitivity of IHA and IFA tests. Table 3 indicates the specificity obtained with each test and summarizes the results observed when *R. typhi* or *R. rickettsii* antigen was used in each test against antisera from known, but different, rickettsial infections. Acute or early convalescent epidemic and murine typhus antisera tested by the IHA procedure showed 86% positive reactors compared with 88% detected with the IFA test. Similar sera from spotted fever patients tested with the IHA reagent indicated 74% positive

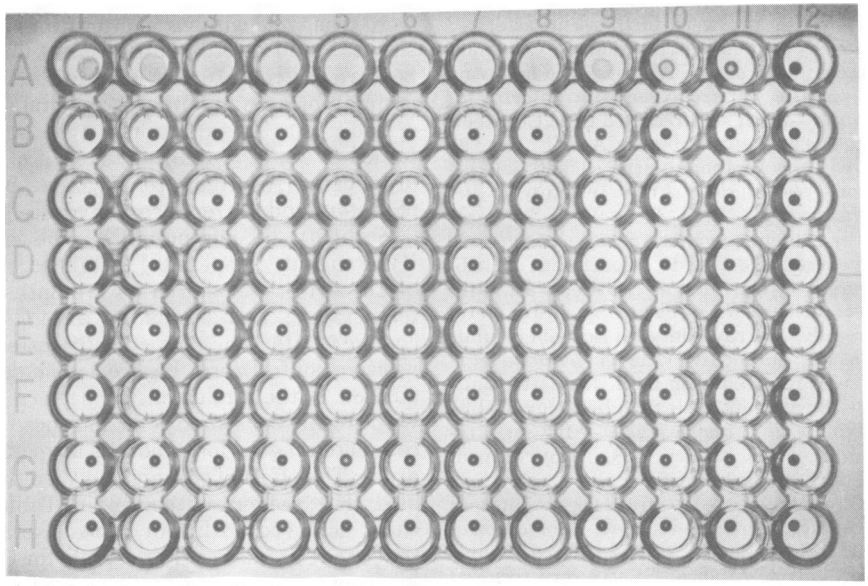

FIG. 1. *Indirect hemagglutination test using glutaraldehyde-stabilized erythrocytes sensitized with ESS from R. typhi. (A) Murine typhus antiserum; (B) epidemic typhus antiserum; (C) Brill-Zinsser disease antiserum; (D) Rocky Mountain spotted fever antiserum; (E) Q fever antiserum; (F) scrub typhus antiserum; (G) normal serum; (H) glutaraldehyde-stabilized sensitized erythrocytes stored at room temperature for 3 months and tested againt same murine typhus serum as in (A). Column 12 is a control for nonspecific agglutination; each well contains a 1:40 dilution of serum combined with nonsensitized stabilized erythrocytes.*

TABLE 1. *Stability of complete IHA reagent after storage at 4 C*

Serum	Antibody titer after									
	0[a]	1	2	3	4	5	6	8	10	12
34-7-9	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40
35-7-9	640	640	640	640	640	640	640	320	320	160
49-4-9	2,560	2,560	2,560	2,560	2,560	2,560	2,560	2,560	1,280	640
50-4-9	10,240	10,240	10,240	10,240	10,240	10,240	10,240	5,120	2,560	2,560

[a] Months of storage.

reactors, whereas 86% were identified by the IFA test. The typhus ESS-sensitized erythrocytes and the spotted fever ESS-sensitized cells were not agglutinated by Q fever or scrub typhus antisera, and no typhus-spotted fever cross-reactions were observed. The IFA test was more sensitive than the IHA test when examining murine and epidemic typhus antisera, with two- to fourfold difference in titer (Table 4). Rocky Mountain spotted fever antisera (Table 5) did not show this constant relationship, and approximately 50% of the sera tested showed higher titers in the IHA than IFA test. In most instances, a rise in titer demonstrated in paired sera by the IFA test was paralleled by similar results in the IHA test. Only three paired sera, from the Rocky Mountain spotted fever group (C.H., H.D., T.M.), showed an increase in titer by the IFA test with total absence of reactivity in the IHA test.

Comparison of sensitivity of IHA, CF, and WF tests. The IHA test was substantially more sensitive than the CF or WF procedure in identifying human sera containing typhus or spotted fever antibodies. Table 6, which summarizes results achieved with each test, indicates that only 57% of the typhus sera were positive by the CF test and 30% by the WF test. Rocky Mountain spotted fever sera showed 62% positive reactors by the CF test and 21% by the WF test. Anti-complementary activity of some sera precluded meaningful results in the CF test, and these sera were not considered as positive. Table 4 indicates that antibody titers observed in typhus sera by the IHA procedure were over 10 times higher than those seen with the CF test,

TABLE 2. *Stability of complete IHA reagent and individual components after storage for 12 months at 4 C*

Serum	Antibody titer		
	Freshly prepared complete typhus IHA reagent	Complete typhus IHA reagent stored for 12 months at 4 C	Typhus ESS and stabilized erythrocytes stored separately for 12 months at 4 C
49-4-9	2,560	640	2,560
50-4-9	10,240	2,560	10,240
204-5-9	40	40	40
205-5-9	640	160	640
358-5-9	320	80	320
209-7-9	320	160	320
38280	5,120	2,560	5,120
38507	2,560	320	2,560
37576	2,560	1,280	2,560
38492	320	80	320
38347	160	<40	160
38488	160	40	160
37416	1,280	320	1,280
38558	2,560	640	2,560
154 (A)	40	40	40
154 (B)	1,280	640	1,280
228 (A)	<40	<40	<40
228 (B)	160	160	160
233 (A)	160	80	160
233 (B)	1,280	640	1,280

and no serum was identified as positive by the CF test that was not also positive in the IHA test. The sensitivity of the WF test in identifying positive sera was poor, but with those sera that were reactive, the WF titers were comparable with IHA titers, and three sera that failed to react in the IHA test were positive by the WF technique.

Rocky Mountain spotted fever sera (Table 5) were much less uniform in their reactivity in the IHA and CF tests. Antibody titers were routinely higher in the IHA test, and 12 sera were identified as positive that were negative in the CF test, but seven other sera were reactive in the CF test and negative by the IHA procedure. Four paired sera (C.E., G.G., B.H., T.L.) showed a rise in antibody titer by the IHA test that was not demonstrable in the CF test. Conversely, three paired sera (C.H., H.D., T.M.) showed a rise in antibody titer by the CF procedure that was not demonstrable by the IHA test. These latter three paired sera are unusual because an increase in antibody titer was demonstrated by both the CF and IFA techniques, but the sera were unreactive in the IHA test. The WF test was the least sensitive in detecting positive sera, but with those sera that were reactive, titers were generally comparable with those seen in the IHA procedure. One

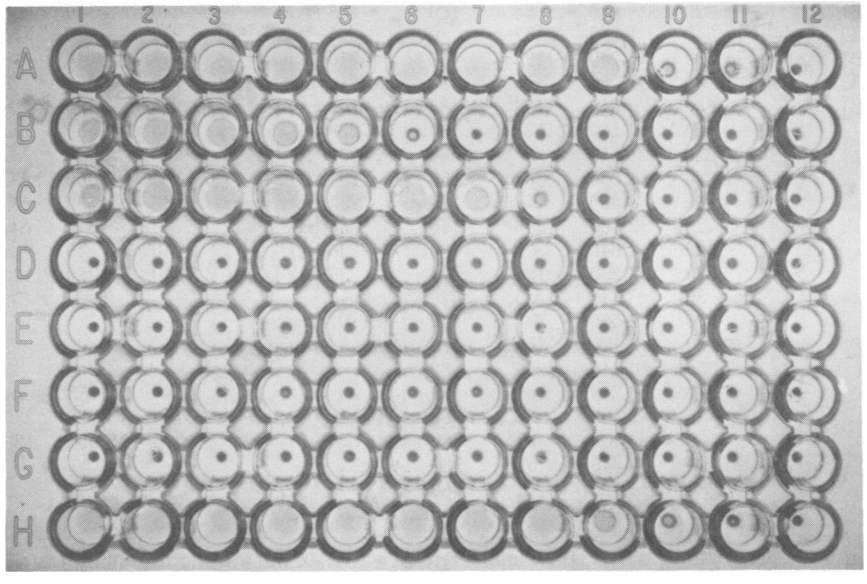

FIG. 2. *Indirect hemagglutination test using glutaraldehyde-stabilized erythrocytes sensitized with ESS from R. rickettsii. (A) Rocky Mountain spotted fever antiserum; (B) murine typhus antiserum; (C) epidemic typhus antiserum; (D) Brill-Zinsser disease antiserum; (E) Q fever antiserum; (F) scrub typhus antiserum; (G) normal serum; (H) diluent only. Column 12 is a control for nonspecific agglutination; each well except 12H contains a 1:40 dilution of serum combined with nonsensitized stabilized erythrocytes.*

TABLE 3. *Detection of rickettsial antibodies in human sera by the IHA and IFA tests*[a]

Serum	IHA		IFA	
	Typhus ESS	Spotted fever ESS	*R. typhi*	*R. rickettsii*
Murine typhus	29/31[b]	0/31	31/31	0/31
Epidemic typhus	15/20	0/20	14/20	0/20
Brill-Zinsser disease	3/3	0/3	3/3	0/3
Rocky Mountain spotted fever	0/42	31/42	0/42	36/42
Scrub typhus	0/102	0/102	0/102	0/102
Q fever	0/16	0/16	0/16	0/16
Normal	0/14	0/14	0/14	0/14

[a] Initial dilution for all sera was 1:40.
[b] Numerator indicates number of positive reactors; denominator indicates number of sera tested.

TABLE 4. *Comparison of typhus group antibody titers in human sera determined by IHA, IFA, CF, and WF tests*[a]

Serum	Titer				Sera	Titer			
	IHA	IFA	CF	WF		IHA	IFA	CF	WF
Murine typhus					Epidemic typhus				
6-7-9 (A)[b]	160	640	32	80[c]					
7-7-9 (B)	640	1,280	AC[d]	640	154 (A)	40	160	<8	<80
34-7-9 (A)	<40	80	<8	160	154 (B)	1,280	1,280	128	<80
35-7-9 (B)	640	2,560	64	80	176 (A)	<40	<40	<8	<80
49-4-9 (A)	2,560	640	<8	160	176 (B)	1,280	1,280	64	<80
50-4-9 (B)	10,240	2,560	64	80	179 (A)	80	160	AC	<80
105-4-9 (A)	2,560	2,560	AC	<80	179 (B)	320	2,560	AC	<80
106-4-9 (B)	1,280	2,560	AC	160	191 (A)	<40	<40	<8	<80
200-7-9 (A)	<40	80	<8	160	191 (B)	640	1,280	32	<80
201-7-9 (B)	320	640	64	160	203 (A)	<40	<40	<8	<80
518-6-9 (A)	640	1,280	32	80	203 (B)	1,280	1,280	256	<80
519-6-9 (B)	1,280	1,280	128	<80	210 (A)	40	<40	<8	<80
204-5-9 (A)	40	80	16	<80	210 (B)	5,120	2,560	128	<80
205-5-9 (B)	640	640	128	<80	228 (A)	<40	<40	<8	160
169-4-9	80	160	128	160	228 (B)	160	320	128	80
507-7-9	320	640	AC	2,560	230 (A)	<40	<40	AC	<80
780-7-9	1,280	2,560	64	1,280	230 (B)	160	2,560	AC	<80
349-7-9	160	320	8	320	233 (A)	160	320	AC	<80
670-7-9	5,120	2,560	256	160	233 (B)	1,280	640	AC	<80
358-5-9	320	640	<8	160	236 (A)	40	40	<8	80
209-7-9	320	1,280	128	320	236 (B)	640	1,280	128	<80
164-2-0	80	640	AC	320					
302-2-0	320	2,560	AC	640	Brill-Zinsser disease				
38280 (A)	5,120	5,120	256	<80					
38507 (B)	2,560	10,240	256	<80					
37576 (A)	2,560	10,240	64	<80	Nes	40	80	64	<80
38492 (B)	320	2,560	32	<80	Kap	5,120	5,120	256	80
38347 (A)	160	160	32	<80	Cap	320	2,560	128	<80
38488 (B)	160	640	32	<80					
37416	1,280	5,120	64	<80					
38558	2,560	5,120	64	<80					

[a] Initial dilution of sera for the IHA, IFA, and WF tests was 1:40; the initial dilution for the CF test was 1:8.
[b] Letters A and B indicate sequential bleedings from the same individual, but not necessarily taken during acute and convalescent stages of infection.
[c] WF titers for typhus group antisera were obtained with OX-19; Rocky Mountain spotted fever sera were tested with both OX-19 and OX-2, and the highest titer obtained with either antigen is indicated. All titers are final dilution of the test.
[d] AC, Anti-complementary serum.

TABLE 5. *Comparison of spotted fever antibody titers in human sera determined by IHA, IFA, CF, and WF tests*[a]

Serum	Titer			
	IHA	IFA	CF	WF
Rocky Mountain spotted fever				
W.S. (A)[b]	<40	160	8	80[c]
W.S. (B)	80	640	64	<80
C.H. (A)	<40	<40	8	<80
C.H. (B)	<40	160	128	<80
W.H. (A)	<40	<40	<8	160
W.H. (B)	640	640	16	<80
H.D. (A)	<40	<40	<8	<80
H.D. (B)	<40	80	16	80
T.J. (A)	640	640	16	160
T.J. (B)	320	320	32	80
L.H. (A)	40	40	<8	<80
L.H. (B)	640	320	256	160
C.E. (A)	640	40	<8	80
C.E. (B)	1,280	160	<8	<80
S.C.	<40	1,280	512	<80
J.A.	1,280	320	32	<80
M.T.	2,560	160	8	<80
J.D.	40	40	<8	80
A.F.	2,560	1,280	16	80
G.G. (A)	40	160	<8	<80
G.G. (B)	80	40	<8	<80
B.H. (A)	<40	<40	<8	<80
B.H. (B)	80	160	<8	80
T.L. (A)	40	<40	<8	<80
T.L. (B)	160	80	<8	<80
T.M. (A)	<40	<40	<8	<80
T.M. (B)	<40	160	16	<80
74-015913 (A)	10,240	10,240	128	80
74-023780 (B)	2,560	640	64	<80
74-013331	320	320	32	<80
74-021615	1,280	640	32	160
74-021616	1,280	320	64	<80
74-021619	2,560	1,280	128	<80
74-021620	640	2,560	32	2,560
74-021621	2,560	640	256	640
74-091741	2,560	2,560	128	1,280
74-091963	2,560	2,560	64	640
75-008042	2,560	160	<8	<80
75-008730	<40	320	64	<80
75-009966	160	640	<8	<80
75-010517	640	320	8	160
75-021781	640	320	<8	<80

[a-c] See Table 4.

serum was identified as positive that was unreactive in the IHA test.

Reaction with animal sera. When erythrocytes sensitized with murine typhus ESS were incubated with rat serum having a complement fixation titer of 1:64 against *R. typhi*, no agglutination was detectable. Attempts to enhance agglutination by rodent sera included: (i) addition of 1% normal rabbit serum as a stabilizing agent; (ii) extraction of immune serum with acetone; and (iii) adjustment of the pH of the reaction mixture from 6.0 to 8.0 in 0.2 pH increments. None of these techniques was successful in obtaining reproducible agglutination of sensitized erythrocytes by rodent sera. Similar results were obtained with immune sera from guinea pigs and rabbits, although occasionally an individual serum did produce interpretable agglutination patterns with the standard technique.

DISCUSSION

This study clearly indicates that glutaraldehyde-stabilized erythrocytes treated with ESS

TABLE 6. *Comparison of serological tests for detection of typhus and spotted fever antibodies in human sera*

Serum	Test			
	IHA	IFA	CF	WF
Murine typhus	29/31[a]	31/31	21/31	15/31
Epidemic typhus	15/20	14/20	7/20	1/20
Brill-Zinsser disease	3/3	3/3	3/3	0/3
Rocky Mountain spotted fever	31/42	36/42	26/42	9/42

[a] Numerator indicates number of positive reactors; denominator indicates number of sera tested.

derived from typhus and spotted fever group rickettsiae were specifically agglutinated by homologous antibody in human sera. The sensitized erythrocytes were stable at room temperature for 3 months and at 4 C for 6 months, but with continued storage antigen deterioration occurred, and after 12 months at 4 C the complete IHA reagent evidenced a two- to fourfold reduction in antibody titers. This problem can be circumvented by storing the ESS and stabilized erythrocytes separately and sensitizing the erythrocytes just before performing the test. Under these conditions, erythrocytes and ESS may be stored for at least 12 months and the complete IHA reagent prepared at that time produces identical titers to those achieved with fresh reagent. The test was group specific and no group cross-reactions were observed with the limited number of sera available. The IHA test, like the IFA procedure, does not allow differentiation of infections within the typhus group, since erythrocytes sensitized with antigen from *R. typhi* were agglutinated by sera of patients with epidemic typhus, murine typhus, and Brill-Zinsser disease.

When sera were used at a screening dilution of 1:40, the IHA test was less sensitive than the IFA in identifying positive reactors, particularly in spotted fever infections. We feel that the practical advantages of this IHA test compensate for its reduced sensitivity, particularly for epidemiological studies conducted in remote areas without access to the sophisticated equipment necessary for the IFA test. The IHA procedure is simple to perform, requiring little equipment and only rudimentary technical training. The development of a reagent easily and economically prepared, with a long shelf life and minimal refrigeration requirements, will allow immediate on-site serological testing by field teams with the possibility of subsequent bleedings from individuals found to be of interest through this IHA screening test.

The results of our comparison of the IHA test with the CF and WF tests suggested that the IHA procedure was more sensitive than the other two tests and evidenced higher titers than the CF test, although evaluation of the CF test was limited by the anti-complementary activity of some sera in our collection. Two earlier studies concerned with a similar comparison, but using unstabilized erythrocytes for the IHA test, support our findings. In one instance (10), concerning 35 patients with murine typhus or Rocky Mountain spotted fever, 31 sera were positive by IHA and 35 were positive by a modified CF test using increased amounts of antigen. Only 13 sera were positive by the WF test. In the second instance (5, 6), concerning 28 patients with epidemic typhus, Brill-Zinsser disease, and Rocky Mountain spotted fever, 28 sera were positive by the IHA test and 27 were positive by a standard CF technique. Only 22 sera were positive by the WF test, and no positive reactors were seen among Brill-Zinsser disease patients. When testing acute and early convalescent sera, both studies indicated that the IHA procedure gave titers approximately 10-fold greater than seen in the CF test.

The principal disadvantage of the IHA test is its inability to regularly detect antibodies in animal sera. Rat sera, demonstrated to contain murine typhus antibodies by the CF test, were completely nonreactive in the IHA test even after several modifications of experimental conditions. Guinea pig and rabbit sera similarly shown to possess murine typhus antibodies were only rarely reactive in the IHA test. Other investigators (6, 7) have also reported substantial inconsistencies when testing guinea pig immune serum with sensitized unstabilized erythrocytes. Studies on the applicability of the IHA test to animal sera are being continued in order to broaden the epidemiological usefulness of this test.

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