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9. ABSTRACT

The sensitivity of Plasmodium knowlesi antigen to human malaria species has been found to be 91% for P. vivax and 86% for P. falciparum. Further evaluation has shown that the sensitivity of the P. knowlesi antigen for eluates of filter paper from individuals infected with human malaria species was 82%. The titer response of an antigen with a serum is a measure of its reactivity. The reactivity of P. knowlesi antigen with P. vivax sera is almost the same as that of P. vivax antigen. With some P. falciparum sera, however, P. knowlesi antigen produces lower titers than those obtained with P. falciparum antigen. In geographic areas where P. falciparum is the predominant malaria species, we noted a decrease in sensitivity of the indirect microhemagglutination (IMHA) test, particularly with sera from children. In the belief that this may be due to the decreased reactivity of the P. knowlesi antigen with P. falciparum sera, an attempt was made to evaluate a mixture of P. knowlesi and P. falciparum antigen to see if the titers obtained with the mixture of cells were higher. When the cells sensitized with the two antigens were mixed in a 1:1 ratio, most of the titers of P. vivax sera were lower or equal to the titers obtained with the P. knowlesi antigen, but were markedly higher than those obtained with the P. falciparum antigen. The same trend was true for the P. falciparum sera. The titers were markedly higher than those obtained with the P. knowlesi antigen and slightly lower than those obtained with the homologous antigen.

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Use of Stable Sensitized Cells in an Improved Indirect Microhemagglutination Test for Malaria

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Aldehyde-fixed cells sensitized with *Plasmodium knowlesi* and *P. falciparum* antigens were tested with sera from *P. vivax* and *P. falciparum* infections. Titers were improved by use of a mixture of cells sensitized with the two antigens.

The sensitivity of *Plasmodium knowlesi* antigen to human malaria species has been found to be 91% for *P. vivax* and 86% for *P. falciparum*. Further evaluation has shown that the sensitivity of the *P. knowlesi* antigen for eluates of filter paper from individuals infected with human malaria species was 82% (3). The titer response of an antigen with a serum is a measure of its reactivity. The reactivity of *P. knowlesi* antigen with *P. vivax* sera is almost the same as that of *P. vivax* antigen. With some *P. falciparum* sera, however, *P. knowlesi* antigen produces lower titers than those obtained with *P. falciparum* antigen.

In areas where *P. falciparum* is the predominant malaria species, we noted a decrease in sensitivity of the indirect microhemagglutination (IMHA) test, particularly with sera from children. In the belief that this may be due to the decreased reactivity of the *P. knowlesi* antigen with *P. falciparum* sera, we attempted to evaluate a mixture of *P. knowlesi* and *P. falciparum* antigen to see if the titers obtained with the mixture of cells were higher.

Fresh human type "O" cells were washed and fixed with pyruvic aldehyde overnight. After tanning, the cells were fixed with glutaraldehyde. Details of this procedure are described elsewhere (2). One batch of double aldehyde-fixed cells was sensitized with *P. knowlesi* antigen which was obtained from infected rhesus monkey (2). A second batch was sensitized with *P. falciparum* antigen which was obtained from an infected Aotus monkey by a method similar to that described by Rogers et al. (5).

All of the positive sera were submitted to the Center for Disease Control, Atlanta, Ga., by various military organizations or state health laboratories. These samples were selected from slide-diagnosed cases of malaria. In all, there were 13 sera from patients with *P. vivax* and 13 sera from patients with *P. falciparum* infection. All negative control sera were obtained from employees of the Center for Disease Control who had no history of malaria infection or of travel outside of the United States. The IMHA test was performed by microtitration in plastic plates. The sera were tested against cells sensitized with (i) *P. knowlesi* antigen, (ii) *P. falciparum* antigen, and (iii) a combination of the two mixed in a ratio of 1:1. The results are shown in Table 1. Two *P. vivax* sera (no. 9 and no. 139) had the same titers with both antigens, but the remaining 11 sera had higher titers with the *P. knowlesi* antigen. One of the *P. falciparum* sera (no. 6) had the same titer with both antigens, but the other 12 sera had higher titers with the *P. falciparum* than with *P. knowlesi* antigen.

When the cells sensitized with *P. knowlesi* and *P. falciparum* antigens were mixed in a 1:1 ratio, most of the titers of *P. vivax* sera were lower or equal to the titers obtained with the *P. knowlesi* antigen but were markedly higher than those obtained with the *P. falciparum* antigen. The same trend was true for the *P. falciparum* sera. The titers were markedly higher than those obtained with the *P. knowlesi* antigen and slightly lower than those obtained with the homologous antigen.

Titers of some sera with the 1:1 antigen were lower than the titers obtained with homologous antigen (e.g., no. 59 and 66 for *P. vivax* and no. 15 and 29 for *P. falciparum*), but the titers improved considerably over the heterologous antigen. Thus, the use of 1:1 antigen for serum samples having antibodies for *P. vivax* and *P. falciparum* is superior to the use of *P. vivax* or *P. falciparum* antigen alone.

TABLE 1. *IMHA titers of serum from patients with malaria infection and normal controls*[a]

CDC[a] code no.	Slide diagnosis	*P. knowlesi* antigen	*P. falciparum* antigen	*P. knowlesi-P. falciparum* (1:1)
139	*P. vivax*	4,096	4,096	4,096
9	*P. vivax*	256	256	512
10	*P. vivax*	4,096	512	4,096
4	*P. vivax*	512	16	256
36	*P. vivax*	2,048	128	1,024
59	*P. vivax*	1,024	64	256
117	*P. vivax*	512	16	256
52	*P. vivax*	512	128	512
66	*P. vivax*	4,096	64	1,024
77	*P. vivax*	256	16	128
99	*P. vivax*	1,024	16	256
S-7	*P. vivax*	2,048	64	1,024
97	*P. vivax*	256	64	128
6	*P. falciparum*	256	256	256
8	*P. falciparum*	128	4,096	2,048
15	*P. falciparum*	32	1,024	256
29	*P. falciparum*	16	512	64
64	*P. falciparum*	128	2,048	256
118	*P. falciparum*	128	4,096	1,024
132	*P. falciparum*	64	1,024	1,024
167	*P. falciparum*	256	1,024	512
57	*P. falciparum*	32	1,024	128
137	*P. falciparum*	64	512	256
S-A	*P. falciparum*	256	4,096	1,024
S-Be	*P. falciparum*	256	4,096	1,024
S-8	*P. falciparum*	256	4,096	1,024
	Control sera[b]	0 (9)[c]	0 (10)	0 (13)
		2 (5)	2 (6)	2 (3)
		4 (1)	8 (1)	8 (1)
		8 (2)		

[a] CDC, Center for Disease Control.
[b] Titers less than 16 are considered negative (5).
[c] Numbers in parentheses refer to the number of sera tested.

There have been reports indicating some cross-reactivity between human and simian plasmodia with the IMHA test. For example, *P. cynomolgi* antigen has been reported to give high titers with *P. vivax* sera and low titers with *P. falciparum* sera. On the other hand, *P. coatneyi* antigen gave high titers with *P. falciparum* sera and low titers with *P. vivax* sera (6). Some of our findings are in agreement with Mahoney et al. (4), who used *P. knowlesi* antigen and obtained higher titers with *P. vivax* than with *P. falciparum* sera. Cross-reactions between human and simian malaria have also been reported by Collins et al. with the fluorescent-antibody test (1).

A previous study in this laboratory (2) has shown that stable sensitized cells can be stored at 4 C for several months. Since a majority of the positive sera received for malaria diagnosis is from either *P. vivax* or *P. falciparum* infections, the use of a combination of the cells sensitized with *P. knowlesi* and *P. falciparum* antigen will increase the reactivity of the IMHA test for detecting malaria antibodies to *P. falciparum*. Furthermore, the use of stable sensitized cells will reduce the routine daily work required for the IMHA test and may improve the reproducibility of the test (2).

LITERATURE CITED

1. Collins, W. E., G. M. Jeffrey, E. Guinn, and J. C. Skinner. 1966. Fluorescent antibody studies in human malaria. IV. Cross-reactions between human and simian malaria. Amer. J. Trop. Med. Hyg. **15**:11-15.
2. Farshy, D. C., and I. G. Kagan. 1972. Use of stable sensitized cells in indirect microhemagglutination test for malaria. Amer. J. Trop. Med. Hyg. **21**: 868-872.

3. Kagan, I. G. 1972. Evaluation of indirect hemagglutination test as an epidemiological technique for malaria. Amer. J. Trop. Med. Hyg. **21**:683-689.
4. Mahoney, D. F., B. C. Redington, and M. J. Schoenbechler. 1966. The preparation and serologic activity of plasmodial fractions. Mil. Med. **131**:1141-1151.
5. Rogers, W. A., Jr., J. A. Fried, and I. G. Kagan. 1968. A modified, indirect microhemagglutination test for malaria. Amer. J. Trop. Med. Hyg. **17**:804-809.
6. Stein, B., and R. S. Desowitz. 1964. The measurement of antibody in human malaria by formalized tanned sheep cell hemagglutination test. Bull. W.H.O. **30**:45-49.