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TERMINAL RESEARCH REPORT
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"Serologic Diagnosis of Malaria"

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II

TERMINAL RESEARCH REPORT

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A. General Background

The field techniques for epidemiologic evaluation of malaria programs on which operational decisions are based consist of blood and spleen surveys, detection of fever cases, and identification of malaria parasites in stained blood films. Epidemiologic surveillance depends upon obtaining blood slides from individuals with fever. But not all patients with malaria have fever; patients with symptoms frequently do not interpret their symptoms as severe enough to warrant attention, and this attention may not be available at the right moment.

The microscopic examination of blood slides is still the only parasitologic laboratory method used in malaria programs to determine the occurrence of malaria and to measure the effect of antimalaria activities. This method can only indicate the presence or absence of patent parasitemia at the time of examination; it does not indicate the individual's malaria experience. Furthermore, the absence of patent parasitemia can be misleading, since patency is influenced by immune status and the use of antimalarial drugs and often occurs only intermittently during malaria infections.

Microscopists in malaria programs examine millions of blood slides each year. In areas with a low incidence of infection, few positive blood films are found, and more sensitive epidemiologic tools are needed. Even in regions with a high incidence of malaria, where determination of spleen sizes could indicate the level of endemicity, a more specific method is required since splenomegaly is a nonspecific physical finding and may be due to causes other than malaria. To provide an improved epidemiologic tool, we have concentrated on the development of serologic methods to detect the presence of antibodies against malaria.

B. Project Objectives

The main objective of the project was to develop simple, rapid, accurate methods for the serologic diagnosis of malaria for use in malaria programs. Diagnostic techniques based on malaria serology were known in principle, but were not developed for field use to meet the large scale case finding and investigational needs of malaria programs. The use of the indirect hemagglutination (IHA), indirect fluorescent antibody (IFA), and complement

fixation (CF) tests and other serologic techniques had to be examined.

Another objective was to produce antigens from monkey and human malarias and to evaluate the sensitivity and specificity of such antigens for the diagnosis of human malaria.

The third objective was to determine the operational use of serologic techniques for epidemiologic and diagnostic purposes.

C. Continued Relevance of Objectives

The difficulties experienced by malaria programs in many countries have emphasized the need for more effective epidemiologic tools to determine the occurrence of malaria. In 1970 the World Health Organization Expert Committee on malaria recommended that "studies be carried out for developing improved parasitological and serological techniques, which will assist in epidemiological assessment" (WHO Techn. Rep. Ser. No. 467, 1971).

In addition, serologic techniques can be more useful than the microscopic method in the investigation of transfusion malaria and other types of malaria in the United States.

D. Accomplishments to Date

1. Findings

a. IHA test

The results of our research suggest that the indirect hemagglutination (IHA) test can be of great practical use as an epidemiologic tool. The laboratory aspects of the development of the IHA test have been emphasized during the Participating Agency Service Agreement (PASA) contract period but some studies could also be conducted to investigate the operational potential of the IHA test.

The antigens used in the IHA test are extracts of *Plasmodium knowlesi* obtained from infected rhesus monkeys. This *Plasmodium* species was chosen because rhesus monkeys can readily be infected, and they provide a large volume of parasitized red blood cells for

antigen extraction. Each batch of antigen is titrated against a set of positive and negative control sera to insure its sensitivity, specificity, and reproducibility. If the antigen qualifies, it is used to sensitize human "O" erythrocytes. The human cells are treated with tannic acid and then sensitized with the soluble antigen. Substituting human cells for sheep cells improved the sensitivity of the test whereas the specificity of the test was increased by using 5 percent normal rabbit serum to saturate the antigenic sites on the red cell after sensitization with antigen and before use in the test. Each lot of sensitized cells is tested again against a small set of positive and negative test sera.

The procedure of the test is fairly simple: serum or filter paper eluates are diluted in microtitration plates, and a drop of antigen-sensitized red blood cells is added; after 1 hour the presence or absence of agglutination is noted.

Because the reactivity of different antigen batches can be quite variable and also because of the instability of the antigens, extensive testing against control sera is required. This is a time- and material-consuming process. Efforts to standardize the antigen by purification have thus far been unsuccessful.

Studies have been carried out in which fresh human red cells were stabilized by fixation with pyruvic aldehyde, treated with tannic acid, and fixed with glutaraldehyde. The cells were then sensitized with either P. knowlesi antigen, P. falciparum antigen, or a combination of these two antigens. Such fixed sensitized cells have remained active for several months. If further evaluations confirm these results and when antigen batches of reproducible reactivity can be developed, the test can be used more widely since the fixed cells are stable when refrigerated. Fixed sensitized cells are being produced commercially by several pharmaceutical companies for other serologic tests, and if a market for malaria antigen were available, antigen could be prepared commercially.

When titers of 1:16 or greater are considered as positive, the sensitivity of the test ranges from 86 to 94 percent and the specificity is 99 percent. When fresh cells were used, 76 percent of the titers of 560 paired sera varied less than two dilutions.

The use of filter paper to collect, store, and ship blood specimens for IHA testing has been evaluated and shown to be of great practical value. The disadvantage is a loss of sensitivity of about 10 percent.

Antibodies can be detected 8 to 27 days after the onset of parasitemia and they can persist for many years. The presence of detectable antibodies and their titer levels seem to be related to the frequency of antigenic stimulations.

Some studies have explored the operational use of the IHA test as an epidemiologic tool. Although few of these studies could be conducted under properly controlled conditions, their results suggest that use of a relatively simple, economical, and reproducible serologic method can be of great practical importance. Some of our studies tried to assess the use of the IHA method for geographic delineation of malaria transmission, to determine the effect of anti-malaria campaigns, and to compare the levels of malaria transmission in different areas.

b. IFA test

The indirect fluorescent antibody (IFA) laboratory has developed a technique to use in making thick smear antigens for detection of antibodies to malaria. This technique has now been adopted by most laboratories which use the IFA test for malaria research.

The sensitivity and specificity of the test were determined, and malaria antibody patterns in patients who had been treated were followed. All of the patients had acquired their infection in Vietnam. Some of them did not develop detectable antibodies to malaria until several days after the onset of illness which suggests that circulating antibody represents a response to parasitemia. It was also noted that antibody levels

decline rapidly within the first 6 months after radical cure. Patients with a history of previous malaria had higher antibody titers than patients without a history of previous malaria attacks. Also, the antibody decline after treatment was more rapid in patients who had no previous record of malaria.

In another study, in which the antibody titers were compared to the infecting Plasmodium species, it was found that the highest antibody titer matched the infecting species in 90 percent of the cases. To increase the sensitivity of the test, we used several Plasmodium species as antigen. In some instances the antibody reacted only with the homologous (the infecting species) antigen. With thick smear antigens containing schizonts, IgM malaria specific antibodies were measured in persons with primary infections and also in infected individuals in malaria endemic areas.

The need for multi-species antigen limited the number of sera that could be tested. To circumvent this problem, a tri-species antigen was prepared from P. falciparum, P. vivax, and P. brasilianum in equal quantity. Evaluation of this antigen showed that there was no reduction in antibody titers as compared with the titration results when the constituent species were used separately. This finding has reduced the time required for testing and increased the usefulness of the IFA test as an epidemiological tool.

c. CF test

With the introduction by Fife of an antigen for the complement fixation (CF) test for malaria, another sero-diagnostic tool became available. Preliminary tests have indicated that the sensitivity and specificity of this method is comparable to that of the IFA test, but evaluations of antigens to identify species by CF have not yet been made. Comparisons of procedures are in progress to assess the use of the CF test in diagnostic and epidemiologic studies.

2. Interpretation of Data and Supporting Evidence

a. IHA test

1) Antigen studies

a) Preparation of IHA antigen

The antigen which we have developed for the IHA test is prepared from *P. knowlesi* maintained in rhesus monkeys (*Macaca mulatta*). Splenectomized rhesus monkeys are infected with the *Anopheles hackeri* strain of *P. knowlesi*, and parasites are counted in blood films made daily. When the parasite count exceeds 10 percent (preferably 50 to 75 percent) of the red blood cells, the monkey is exsanguinated when the parasites are in schizogony. Blood from a 3-kg monkey can yield 10 to 40 ml of plasmodia, which are frozen at -70° C or lower.

For use in the test, frozen parasites are thawed in tepid water, diluted 1:3 to 1:5 in diluent (equal volumes of pH 6.4 phosphate buffer and 4 percent NaCl), and passed through a Ribi Press operated at 17,000 pounds per square inch (psi). The use of the Ribi Press has improved the antigen stability because the heat denaturation of antigen is less than in the poorly cooled French pressure cell which was previously used. The material from the Ribi Press is centrifuged and the resulting supernatant fluid is the stock antigen. The optimal dilution of antigen is determined by titration against a battery of sera with known titers. Prior to rupture, parasites maintain their active antigens for periods up to 9 months at -70° C. However, once freed from the parasite, the antigens are quite labile and lose activity after 24 hours at 4°C and after 36 to 48 hours at -20° C; at -70° C they are stable for only about 1 month. Lyophilization of intact parasites results in only a slight loss of reactivity and pattern resolution whereas lyophilization of the stock antigen completely destroys the reactivity.

Because the ruptured antigens presently in use are extremely labile, it is impossible to make large amounts of antigens or to prepare antigens for use in other laboratories; therefore, vigorous efforts have been made to extract active antigens in a more stable form.

A number of workers have reported partially purified Plasmodium antigens which they found to be sensitive, specific, and stable in several different types of serologic tests. D'Antonio et al. (PSEBM:125, 1966) reported a complement fixing antigen prepared by gel filtration of pressure disruptured parasites. Antigens prepared from P. knowlesi and P. falciparum by this method were found to have good specificity and sensitivity in CF tests (D'Antonio et al., Milit. Med. 131 Suppl.: 1152, 1966). Wellde et al. (Milit. Med. 134: 1284, 1969) described a procedure for extracting antigens from lysates of infected cells by ammonium sulfate precipitation followed by gel filtration on Sephadex G-200 or ion exchange chromatography. Each of these methods, singly and in combination, has been tested in our laboratory. Using P. knowlesi from rhesus monkeys and P. falciparum from Aotus monkeys, we carried out 40 unsuccessful experiments in which we attempted to produce a suitable antigen by these methods. The antigens we isolated using these methods were somewhat more stable than our present antigen; but much of the antigenic activity was lost. Limited success has been achieved using modifications of the rivanol precipitation method of Chavin (Milit. Med. 121 Suppl.: 1124, 1966). Active antigens have been isolated by rivanol precipitation from disrupted parasites, plasma, and infected erythrocytes. Serum soluble antigens derived from P. knowlesi infections in some rhesus monkeys were evaluated for use in the IHA test. At least three antigen components have been identified by gel diffusion analysis, and two of these are precipitated by rivanol. This rivanol-insoluble complex sensitizes red cells and is active in the IHA test.

Rivanol precipitation of disrupted infected red cells can isolate an antigen complex which is serologically active in the IHA test but does not have sufficient sensitivity to replace antigens presently in use. The rivanol insoluble antigens have proved difficult to work with because there is no reliable method for determining the best sources of these antigens. The factors in the host-parasite relationship which determine the location of these antigens in plasma, the red cell, or the parasite are not clear. Exhaustive testing of each component is necessary, but the enhanced stability of these antigens makes continued work on them worthy of attention. The test results of some of the experiments are given in Table 1.

In addition to P. knowlesi antigens, serum soluble antigens of P. gallinaceum prepared by Dr. M. Ristic of the University of Illinois have been evaluated. Some of these antigens appear to have a low sensitivity in detecting antibodies in vivax infections (Table 2). Other antigens of this type will be evaluated when available. We have provided material from P. knowlesi-infected monkeys to Dr. Ristic who is attempting to fractionate it by his methodology (Lykins et al., Am. J. Trop. Med. Hyg., 20: 394, 1971).

b) Stabilization of the red cell carrier

Human "O" cells were stabilized with pyruvic aldehyde and glutaraldehyde, separated by the tanning procedure. These cells were then sensitized with a P. knowlesi antigen. This antigen was prepared by sonification in a Biosonik II A for 7 minutes. Forty sera were tested monthly over a period of 120 days. Thirty-five sera came from persons who had had a parasitemia and five sera were obtained from persons who had never been exposed to malaria. Only one of the 160 positive replicate test results differed by more than one dilution from the most frequently found titer level (Table 3).

TABLE 1

Examples of Antigen Stabilization Experiments*

	Sera				
A. Lyophilization of Plasmodia					
	R.	T.	M.	Mk.	
a) standard antigen	0	6-7	7-8	12+	
b) Plasmodia lyophilized, reconstituted and pressed	0	5-6	6-7	11-12	
c) stock antigen lyophilized	0	0	0	0	
B. Chromatography -Sephadex G-200					
	F.	T.	M.	Mk.	
Standard antigen, lysate dilut. 1:4	0	4	6	7	
Tube 28 undilut.	0	0	0	1-2	
Tube 55 undilut.	2	0	2-3	4	
Tube 66 undilut.	0	0	0	3	
C. Rivanol Extraction					
	R.	J.	B.	E.	Mk.
a) M 131 antigen 1:4	0	8-9	8-9	7	11-12
b) M 131 antigen 1:8	0	8-9	7-8	6-7	9-10
c) Rivanol prep. from M 131 plasma	All cells lysed at 1:3 dilution				
d) Rivanol prep. from M 131 lysis water concentr. to 50% of original volume	0	5-6	7	6	11-12

*Results recorded in dilution endpoints

TABLE 2

Experiment with *Plasmodium gallinaceum* Antigens*

Sera	Antigens					
	P. knowlesi	*P. gallinaceum*				
		I	II	III	IV	V
K (not infected)	0	0	0	11	1-3	11
J (*P. vivax*)	8-9	0	0	1	0	3
B (*P. falciparum*)	8-9	9	8	5	6	8
E (*P. ovale*)	6-8	9	7	9	5	12
J (*P. vivax*)	9-11	0-7	3	7	9	7
Monkey (*P. knowlesi*)	6-7	0-1	0-3	0-2	4	3

*Results recorded in dilution endpoints

Table 3

IHA Titers of Sera With Stable Sensitized Cells

No.	CDC Code	Days After Preparation					No.	CDC Code	Days After Preparation				
		0	30	60	90	120			0	30	60	90	120
1	69	32	32	32	32	32	21	166	1024	1024	1024	1024	2048
2	66	4096	4096	4096	4096	4096	22	174	512	256	256	256	128
3	73	0	4	0	0	0	23	146	512	512	256	128	128
4	74	0	0	0	0	0	24	152	1024	2048	1024	2048	2048
5	75	256	128	256	128	64	25	149	512	512	512	512	512
6	76	128	128	256	128	128	26	162	512	512	256	256	256
7	77	256	512	256	256	128	27	173	0	0	0	0	0
8	91	128	64	128	64	64	28	181	1024	1024	1024	1024	2048
9	93	4096	4096	4096	4096	4096	29	178	8	8	8	0	4
10	94	0	0	0	0	2	30	176	4096	2048	4096	4096	4096
11	34	256	128	128	128	64	31	116	512	256	512	256	256
12	33	32	16	16	16	16	32	145	4096	4096	4096	4096	4096
13	10	4096	4096	4096	4096	4096	33	114	4096	4096	4096	4096	4096
14	29	128	64	128	128	32	34	113	0	0	0	0	0
15	57	512	256	256	256	128	35	99	256	512	512	512	512
16	35	0	0	0	0	0	36	98	4096	4096	4096	4096	4096
17	52	2048	4096	4096	4096	4096	37	135	4096	4096	4096	4096	4096
18	53	128	256	128	128	64	38	137	1024	1024	1024	1024	1024
19	37	0	0	0	0	2	39	97	256	512	256	256	128
20	32	256	256	256	256	256	40	139	4096	4096	4096	4096	4096

In another experiment, three batches of double aldehyde fixed cells were sensitized with 1) a P. knowlesi antigen, 2) a P. falciparum antigen obtained from an Aotus monkey, and 3) a 1:1 mixture of the two antigens. The titers of sera from falciparum infections were higher with the P. falciparum antigen and with the antigen mixture, than with the P. knowlesi antigen (Table 4). However, some of these falciparum sera had been tested previously with another P. knowlesi antigen (Table 5) and the titers were the same or higher, even when compared with the antigen mixture. This suggests that the reactivities of the P. knowlesi antigens used in the two experiments were not readily comparable.

Subsequently, 3,700 serum samples were tested for further evaluation of the double aldehyde fixed cell as an antigen carrier in the IHA test. These samples included sera from residents of Haiti, Honduras, Brazil, and New Guinea; sera from participants in malaria drug studies and from individuals without a history of travel outside the United States; and sera with positive titers to malaria when tested with the IFA method. In these experiments, the sensitivity varied from 91 to 94 percent in sera from persons with vivax infections and ranged between 70 and 91 percent in sera from persons with falciparum infections. The specificity varied from 90 to 98 percent. When sera were tested with double aldehyde fixed cells sensitized with different lots of antigen, the reproducibility was much lower than when a single antigen lot was used. These experiments indicate that the use of fixed cells permits testing of a large number of sera over a long period of time with a single antigen lot with highly comparable test results. When different antigen lots are used, their reactivity has to be adjusted by testing with a large number of positive and negative control sera to ensure comparability.

Table 4

IHA Titers of Serum from Patients with Malaria Infection and Controls

CDC Code	Slide Diagnosis	*P. knowlesi* antigen	*P. falciparum* antigen	*P. knowlesi* *P. falciparum* (1:1)
139	*P. vivax*	4096	4096	4096
9	"	256	256	512
10	"	4096	512	4096
4	"	512	16	256
36	"	2048	128	1024
59	"	1024	64	256
117	"	512	16	256
52	"	512	128	512
66	"	4096	64	1024
77	"	256	16	128
99	"	1024	16	256
S-7	"	2048	64	1024
97	"	256	64	128
6	*P. falciparum*	256	256	256
8	"	128	4096	2048
15	"	32	1024	256
29	"	16	512	64
64	"	128	2048	256
118	"	128	4096	1024
132	"	64	1024	1024
167	"	256	1024	512
57	"	32	1024	128
137	"	64	512	256
S-A	"	256	4096	1024
S-Be	"	256	4096	1024
S-8	"	256	4096	1024
Seventeen Control Sera (Negative)		Nine 0 Five 2 One 4 Two 8	Ten 0 Six 2 One 8	Thirteen 0 Three 2 One 8

TABLE 5

IHA Titers of Sera with Stable Cells Sensitized with Two *Plasmodium knowlesi* Antigens and Antigen Mixture

CDC Code	Slide Diagnosis	Antigen Used in Experiment: Table 3 *P. knowlesi*	Table 4 *P. knowlesi*	Table 4 *P. knowlesi* / *P. falciparum* (1:1)
139	*P. vivax*	4096	4096	4096
10	*P. vivax*	4096	4096	4096
52	*P. vivax*	4096	512	512
66	*P. vivax*	4096	4096	1024
77	*P. vivax*	256	256	128
99	*P. vivax*	512	1024	256
97	*P. vivax*	256	256	128
29	*P. falciparum*	64	16	64
57	*P. falciparum*	256	32	128
137	*P. falciparum*	1024	64	256

2) Sensitivity, specificity, and reproducibility

To determine the IHA titer levels which can be considered indicative of the presence of antibodies to malaria, we made a proportionate frequency distribution of the IHA titer values found in sera of individuals who had never been exposed to malaria and of persons who had a parasitemia at the time of examination (Figure 1). On this basis, sera with IHA titers of 1:8 or greater are considered to be positive, but because the first dilution of filter paper eluates are approximately a 1:16 dilution of serum, this titer level was adopted for routine use as the lower limit of positive reactions.

The specificity (i.e., the proportion of negative reactors in persons who had never had malaria) was measured by testing sera from 2,400 individuals, including military recruits in the United States; Eskimos on St. Lawrence Island, Alaska; tuberculosis and syphilis patients in the United States; and Indians in North Carolina who were heavily infected with intestinal parasites (Table 6). Only nine of these 2,400 individuals (0.4 percent) had a serologic reaction to the P. knowlesi antigen of 1:16 or higher. For a hemagglutination test, the malaria antigen has an exceedingly high specificity which makes it exceptionally valuable for epidemiologic studies.

The sensitivity (i.e., the proportion of positive reactors in individuals with recent malaria infection) was determined by testing 1,202 specimens from patients who were determined by microscopic blood-slide examination to have malaria (Table 7). This group included patients with induced infections in the United States and individuals who had been infected in malaria-endemic areas, such as U. S. military personnel who had acquired their infection in Vietnam, and people living in Brazil, Ethiopia, Haiti, New Guinea, Bangladesh, Honduras, and the Philippines. The sensitivity of the test was 91 percent for vivax infections, 86 percent for falciparum infections, and 94 percent for infections due to P. malariae.

Figure 1

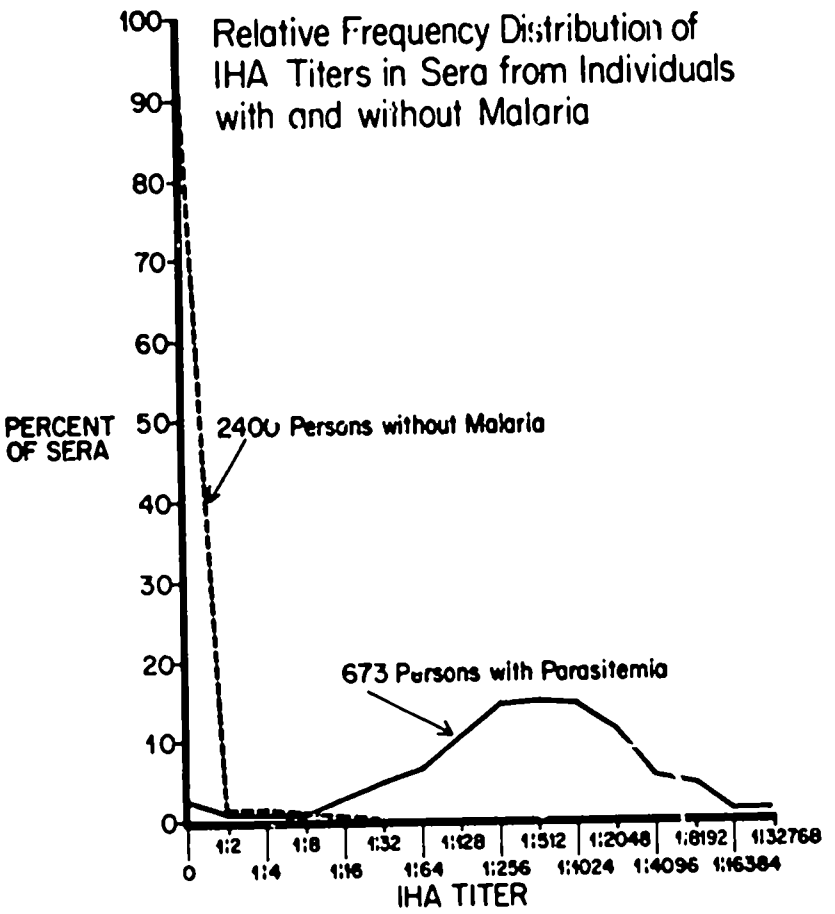

Relative Frequency Distribution of
IHA Titers in Sera from Individuals
with and without Malaria
PERCENT OF SERA
100
90
80
70
60
50
40
30
20
10
0
2400 Persons without Malaria
673 Persons with Parasitemia
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
1:4096
1:8192
1:16384
1:32768
IHA TITER

TABLE 6

Specificity of the IHA Test

Number of Sera Tested	Titer < 1:16 Number	Titer < 1:16 Percent	Source of Specimens
2237	2230	99.7	U. S. recruits
61	61	100.0	Alaska
54	53	98.1	Tuberculosis and Syphilis patients
48	47	97.9	North Carolina Indians *
Total 2400	2391	99.6	

* Forty-four were infected with one or more intestinal parasites.

TABLE 7

Sensitivity of the IHA Test*

	Plasmodium species	Number of sera tested	Response ≥1:16 number	Response ≥1:16 percent	Source of sera
A. Induced infections					
	vivax	19	18	95	U.S.
	falciparum	13	11	85	U.S.
	malariae	2	2	100	U.S.
B. Naturally acquired infections:	vivax	58	48	83	U.S. military from Vietnam
		130	129	99	Honduras
		100	95	95	New Guinea
		19	19	100	Brazil
		62	51	82	Philippines*
		8	7	88	Ethiopia*
		26	24	92	Brazil*
		58	45	78	Bangladesh*
	Total	461	418	91	
	falciparum	17	16	94	U.S. military from Vietnam
		9	8	89	Haiti
		27	27	100	Brazil
		162	147	91	New Guinea
		3	2	67	Pakistan*
		195	154	79	Ethiopia*
		36	35	97	Brazil*
		119	100	84	Philippines*
	Total	568	489	86	
	malariae	117	112	96	New Guinea
		22	19	86	Ethiopia*
	Total	139	131	94	
	TOTAL	1202	1069	89	

*Collected on filter paper

Reproducibility studies of the IHA test showed that within-day variations of one dilution or less were obtained for 88 percent of 235 duplicate serum determinations. Day-to-day variations of one dilution or less were obtained for 76 percent of 560 duplicate serum determinations (Table 8).

3) Filter paper blood collection method

To evaluate a filter paper blood collection method for use in malaria serology, we tested a number of types of absorbent filter paper and selected ROPACO #1024-.038.

The filter paper is cut into 1- x 3-inch rectangles and imprinted with circles of 12 mm diameter. Capillary blood is collected from a finger prick by placing the paper, with the circle uppermost, over the bleeding point and allowing the circle to fill with blood. Approximately 0.1 ml is required to saturate the circle. The papers are dried at ambient temperature, packaged together with glassine interleaves, and kept cool and dry for shipment. For laboratory storage, freezing at -20° C is recommended.

Preliminary studies with paired serum and filter paper blood specimens from infected monkeys indicated that the eluate from the filter paper represented approximately a 1:8 dilution of serum. Filter paper specimens kept dry at temperatures up to 40° C for 15 days and specimens kept at 37° C and 100 percent humidity for 5 days showed only a slight loss of antibody activity; the eluate was equivalent to a 1:11 dilution of serum.

With serum obtained by venipuncture from 673 persons with slide proven parasitemia, the sensitivity of the test was 93.9 percent. With blood collected by the filter-paper absorption method from infected individuals, the sensitivity was 82.6 percent (Table 9).

To obtain a direct comparison of the IHA titers of filter paper blood specimens with those of sera from venous blood, we collected paired specimens by both methods from 335 individuals in Haiti and 70 persons in the State of Mato Grosso, Brazil.

Table 8

Agreement of Duplicate Titrations in the IHA Test for

1968 to 1971

Reagents		WITHIN DAY			DAY-TO-DAY		
Antigen	Cells	Duplicate determinations	Percent differing by ≤ 1 dil.	≤ 2 dil.	Duplicate determinations	Percent differing by ≤ 1 dil.	≤ 2 dil.
Same	Same	185	89	96	96	70	92
Same	Different	-	-	-	96	88	96
Different	Same	50	86	92	24	87.5	100
Different	Different	-	-	-	344	75	90
Total		235	88	95	560	76	91

Same = Antigen from a single monkey or red blood cells from a single donor

Table 9

Sensitivity of the IHA test (≥ 1:16) by method of specimen collection.

Plasmodium species	No. sera tested	Per cent positive
	Serum	
vivax	326	94.8
falciparum	228	91.7
malariae	119	95.8
Total	673	93.9
	Filter paper blood	
vivax	154	82.5
falciparum	353	82.4
malariae	22	86.3
Total	529	82.6

The positive and negative IHA test results of those 405 paired specimens are summarized in Table 10. Both sera and filter paper blood specimens of 203 of the 405 examined individuals (50.1 percent) were positive, whereas 135 of the paired specimens (33.3 percent) had a negative titer.

"False negative" titers, i.e., those that were positive by serum titer but negative by filter paper titer, were found in 58 of 260 (21.9 percent) paired specimens. Also, 10 of 145 (6.8 percent) persons with negative IHA serum titers had positive titers when their filter paper specimens were examined. Sixty-one percent of the paired differences for positive specimens were within one dilution. The corresponding serum titer value was calculated for each filter paper titer value, and it appears that the greatest loss of sensitivity occurs with filter paper specimens from blood with serum titers of 1:16 to 1:32. These specimens are often negative by the filter paper method (Table 11).

4) Development and persistence of antibodies

Sera from individuals who participated in malaria drug studies were examined to determine the development of antibodies. Antibodies were detected with the IHA method 8 to 27 days after the onset of parasitemia in individuals with primary infection.

To determine the persistence of hemagglutinating antibodies after malaria infection, we examined sera or filter paper blood specimens from four different populations for whom the possibility of reinfection could be excluded (Table 12).

One group consisted of 49 Nigerian students in Washington, D. C. When they were examined, they had lived in the United States for continuous periods ranging from 1 month to 7 years. Some students gave a history of symptoms compatable with malaria before departure to the United States, and some had reportedly used chloroquine while still in Nigeria. Examination of blood slides obtained simultaneously with the filter paper blood specimens did not reveal the presence of Plasmodium parasites. Examination of

TABLE 10

Positive* and Negative IHA Titers in 405 Paired Sera and Filter Paper Blood Specimens

		Filter Paper		
		Negative	Positive	Total
Serum	Negative	135	10	145
	Positive	57	203	260
	Total	192	213	405

*Titer $\geq$1:16

TABLE 11

IHA TITERS OF PAIRED SERA AND FILTER PAPER BLOOD SPECIMENS FROM 405 INDIVIDUALS

IHA Titer Serum	IHA Titer - Filter Paper <1:16	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	1:16384	Total
<1:16	135	8	2										145
1:16	33	5	4		1								43
1:32	14	15	11	2	1				1				44
1:64	8	9	15	9	5	1				1			48
1:128	1		4	5	9	4	3	1					27
1:256			3	2	3	7	5	1					21
1:512	1				4	8	3	2	1				19
1:1024					3	5	9	5	4	5			31
1:2048							1	5	1	2	2		11
1:4096								1			2		3
1:8192										1			1
1:16384								1	2	1	2		6
1:32768										4	1	1	6
Total	192	37	39	18	26	25	21	16	9	14	7	1	405
Corresponding serum titer	<1:16	1:19	1:46	1:83	1:167	1:357	1:512	1:1218	1:1287	1:3327	1:6654	1:32768	

61.4% of the paired differences for positive specimens were within 1 dilution.

TABLE 12

Persistence of Hemagglutinating Antibodies to Malaria After Naturally Acquired Infections

Population and Material Tested	Plasmodium Species	Interval*	Number Examined	IHA Titer ≥1:16		Geometric Mean Titer
				No.	%	
1. Camp Fire girls, Lake Verna, Calif. (serum)	P. vivax	13 years	10	10	100	1:49
2. U. S. Military (serum)	P. vivax	0-60 days	121	106	87.6	1:375
		7 months	67	56	83.6	1:75
		12 months	36	27	75.0	1:49
3. Nigerians in U.S.A. (filter paper)	P. falciparum	<6 months	6	6	100	1:1112
		6-11 months	8	7	88	1:343
		1 year	10	9	90	1:405
		2-3 years	18	16	89	1:431
		4-7 years	7	7	100	1:71
4. Patients on Tabago (filter paper)	P. malariae	3 years	27	5	18.5	1:18

*Since treatment (for Group 3 - since arrival in the United States)

their filter paper blood specimens with the IHA test indicated that 92 percent of the students had a positive IHA titer. The geometric mean titer of the reactors declined from 1:1112 for students who had been in the United States for less than 6 months to a titer of 1:71 for the students who had resided in the United States at least 4 years.

Sera were obtained at intervals up to 1 year from military personnel who had served in Vietnam and had been hospitalized at Fort Bragg, North Carolina, for treatment of vivax infections acquired overseas. The patients had usually adhered to a malaria chemoprophylactic regimen while in Vietnam. The diagnosis of vivax malaria was based on finding P. vivax organisms in their peripheral blood, and they were promptly treated at the military hospital after the onset of illness. In this group, 84 percent had a positive IHA titer 6 months after admission to the hospital, and 71 percent still had a positive serologic reaction 12 months later. None of the patients had returned to malaria-endemic areas since their hospitalization for vivax malaria.

Sera were tested from 10 Campfire girls who had acquired vivax malaria (Korean strain) during an outbreak at Lake Vera, California, in 1952; 13 years after the outbreak their IHA serum titers were between 1:32 and 1:128. All of the girls had been treated for malaria and they had not visited malaria-endemic areas between 1952 and 1965, when their sera were obtained.

Filter paper blood specimens were obtained from 27 persons who had acquired a P. malariae infection in Tobago in 1966 when a small outbreak, involving 38 cases, occurred on that island. The cases were promptly treated, and reinfection of these patients seems most unlikely since malaria has not been endemic on the island since 1953. Only 19 percent of the 27 patients had positive IHA titers to malaria 3 years after they had been treated.

In addition, sera were examined from individuals in the United States who had acquired induced malaria. Six of 12 persons who had been infected with P. malariae had IHA titers of 1:16 or greater up to 24 years after radical cure for malaria. Six other individuals had been infected with P. falciparum. Three of these had positive IHA titers from 4 to 16 years after their malaria infection had been terminated.

The presence of detectable antibodies and their titer level may be related to the frequency of antigenic stimuli. Seropositivity rates in patients with patent parasitemia in the Philippines and in Ethiopia were both high and independent of age (Figure 2). In contrast, the geometric mean positive titers in the Philippine patients were relatively low in the younger age groups and rose with increasing age, but were high in all age groups in the Ethiopian patients. The most notable parasitologic difference between the two populations from which the patients came was that the parasite rate in children under 5 years of age was much higher in Ethiopia than in the Philippines (62 percent vs. 7 percent), which suggests that the frequency of malaria infections was greater in the Ethiopian population. A serologic study of healthy U. S. military personnel who had served in Vietnam showed a positive relationship between the number of malaria attacks and the seropositivity rate.

5) The operational use of the IHA test

Studies were conducted to explore the potential operational applications of the IHA test.

a) Sera of military recruits in the United States, Brazil, Colombia, and Argentina were examined with the IHA test. Each collection consisted of approximately 3,000 sera from all parts of each country and represented a cross-section of the young male population inducted into the armed forces (Table 13). Negative titers in 99 percent of the U. S. recruits confirmed the absence of malaria in this country.

FIGURE 2

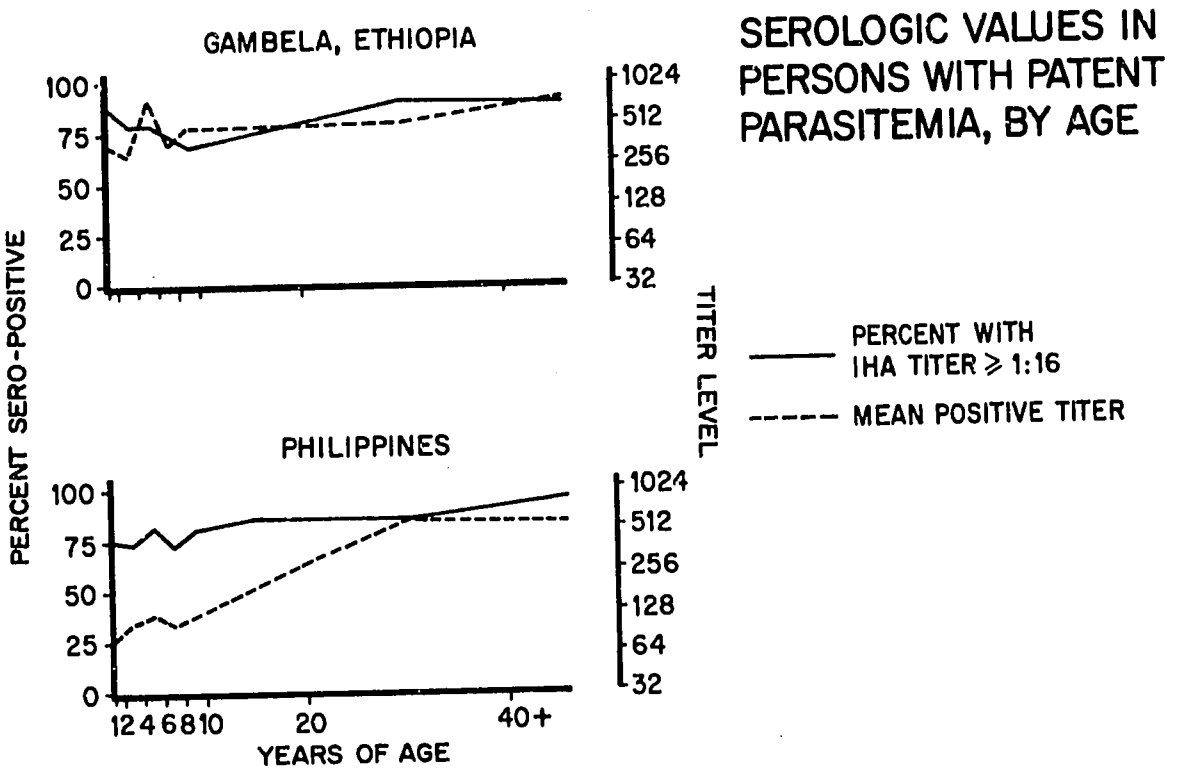

SEROLOGIC VALUES IN PERSONS WITH PATENT PARASITEMIA, BY AGE
GAMBELA, ETHIOPIA
PHILIPPINES
PERCENT SERO-POSITIVE
100
75
50
25
0
TITER LEVEL
1024
512
256
128
64
32
1 2 4 6 8 10
20
40+
YEARS OF AGE
PERCENT WITH IHA TITER ≥ 1:16
MEAN POSITIVE TITER

TABLE 13

MALARIA ANTIBODY TITERS OBTAINED WITH MILITARY RECRUIT SERA

Country	Total	Titers																	
		0	2	3	4	8	9	16	27	32	64	81	128	243	256	512	1024	2048	4096
United States	2237	2203	7		7	13		7											
Brazil	2681	1979	76		68	153		134		129	59		34		27	12	8	2	
Colombia	2961	2162	62		108	134		166		146	93		46		31	8	2	1	2
Argentina	3077	2877		58			100		30			10		2					

In the Brazilian and Colombian recruits, a serologic prevalence rate of approximately 20 percent was observed, whereas the slide positive rate was only 3 to 5 percent. The states having the highest serologic prevalence rate were ranked with states having the highest slide positive rates, and an 80 percent agreement was observed (Table 14). The same correlation was observed in Argentinean recruits; the serologic prevalence was approximately 5 percent, and the slide positivity rate was less than 1 percent.

b) Sera from residents of the Philippines were collected during a WHO survey, and it was noted that on the island of Cotabato the prevalence of malaria IHA antibody ranged from 10 to 56 percent. Two villages with high serologic positivity rates (35 to 56 percent) and high geometric mean reciprocal titers could be found within a group of villages which had seropositivity rates ranging from 10 to 15 percent and with low mean titers. The data suggest a higher level of transmission in the villages with high serologic indices than in the surrounding localities (Figure 3).

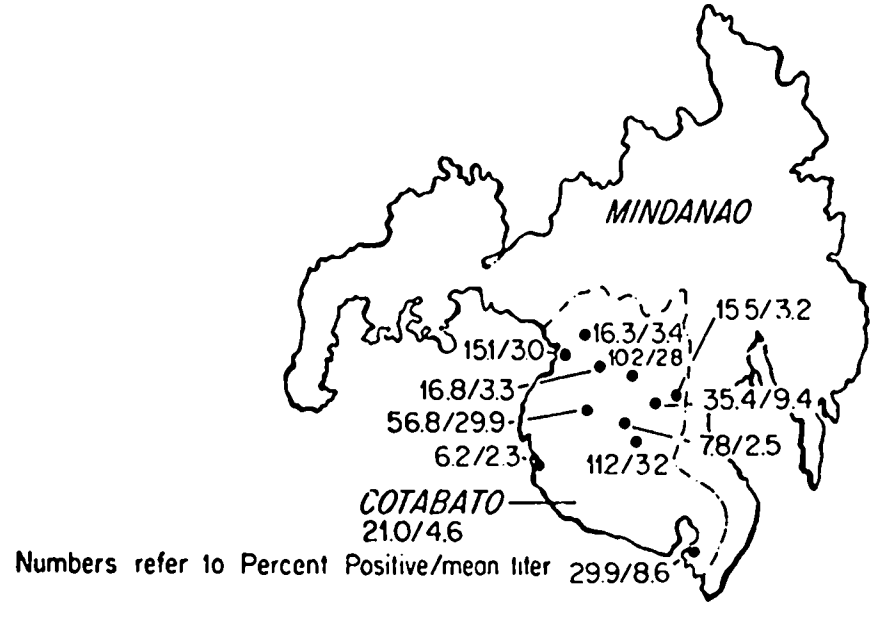
c) In Ethiopia, serum samples taken in two locations in both wet and dry seasons showed that the seropositivity rate and the geometric mean titer increased markedly in the wet season, the time of peak transmission (Table 15). This finding suggests that the IHA test has potential for monitoring seasonal changes in malaria transmission.

d) In Nepal an investigation was conducted to determine whether malaria was transmitted above 4,000 feet. In a cluster of villages above 4,000 feet, 163 sera were collected. Of the 22 individuals who were found to have positive IHA titers, 19 were males with a history of travel into the valley and into a malarious zone; 3 were young women who did not admit to visits to the valley, but women in this area are reportedly reluctant to admit that they have left the village.

TABLE 14 BRAZIL: COMPARISON OF SEROLOGIC AND BLOOD SLIDE RESULTS FOR MALARIA FROM STATES FROM WHICH BOTH DATA WERE AVAILABLE. STATES ARE RANKED FROM HIGHEST TO LOWEST VALUE.

	Serology			Slide examination		
Rank	*State code*	*Name of state*	*Per cent positive*	*State code*	*Name of state*	*Per cent positive*
1	3	Roraima	100	3	Roraima	16.8
2	4	Amazonas	39.8	25	Goias	15.7
3	5	Para	39.3	7	Maranhão	15.5
4	2	Acre	37.5	5	Para	14.1
5	12	Pernambuco	33.6	16	Bahia	8.7
6	7	Maranhão	27.6	8	Piaui	6.6
7	25	Goias	25.6	4	Amazonas	5.5
8	24	Mato Grosso	25.4	2	Acre	4.7
9	13	Alagôas	25.0	18	Espirito Santo	4.6
10	8	Piaui	23.3	24	Mato Grosso	3.5
11	17	Minas Gerais	18.6	17	Minas Gerais	3.4
12	16	Bahia	17.3	22	Santa Catarina	3.3
13	10	Rio Grande do Norte	15.8	9	Ceará	2.3
14	22	Santa Catarina	12.5	21	Paraná	2.0
15	11	Paraiba	8.8	12	Pernambuco	1.3
16	19	Rio de Janeiro	8.3	19	Rio de Janeiro	0.6
17	9	Ceará	4.6	11	Paraiba	0.4
18	21	Paraná	4.3	27	Guanabara	0.3
19	15	Sergipe	0.0	15	Sergipe	0.1
20	18	Espirito Santo	0.0	13	Alagôas	0.0
21	27	Guanabara	0.0	10	Rio Grande do Norte	0.0

FIGURE 3



Distribution of malaria antibodies and geometric mean reciprocal titers for a number of villages in the province of Cotabato on the island of Mindanao, Philippine Republic.

TABLE 15

ETHIOPIA: Seasonal Fluctuation of Malaria Antibody Levels

Location	June 1968 (Dry)		October 1968 (Wet)	
	Percent Positive	Mean Titer	Percent Positive	Mean Titer
Om Hagar School	50.0	1:18.8	65.5	1:30.5
Humera Farms	68.4	1:56.1	78.2	1:113.0

In a study in Ethiopia, only 22 percent of the sera collected from persons living above 6,000 feet were positive, whereas 58 percent of the sera from individuals residing below 6,000 feet were positive.

e) Studies were conducted in February 1970 in two localities in Bangladesh (Table 16). In one of these localities, Balihar, none of the examined children under 8 years of age had demonstrable antibodies against malaria; in the other locality, Kalapara, 23 percent of the children under 2 years old had a titer of 1:16 or greater. Total coverage with DDT spraying in Balihar began in May 1964, and the surveillance data indicate that there had been no transmission of malaria in this locality since 1965. A baseline survey in Kalapara in 1968 showed 124 of 1,315 (9.4 percent) blood slides to be positive.

f) Age specific serologic indices were determined in three areas with different levels of malaria to estimate the intensity and distribution of malaria transmission.

Figure 4 shows the serologic profiles of populations in Palawan, Philippines; in the sector Cuiaba, State of Mata Grosso, Brazil; and in Balihar, Bangladesh. No antibodies were detected in children under 8 years of age in Balihar, Bangladesh, but in adults the seropositivity rates increased to levels comparable to those observed in Palawan and Brazil.

Although the seropositivity rates in adults were lower in Brazil than those in Balihar, the geometric mean titers of the serologic positive reactors in Brazil were higher than those observed in Balihar, suggesting that the adults in Brazil had been infected more recently than those in Bangladesh (Tables 17 and 18).

In the Philippines, the seropositivity rates and the geometric mean titers of the reactors were higher in each age group than those in the two other areas (Table 19). Tables 17, 18, and 19

TABLE 16

MALARIA IN TWO LOCALITIES - BANGLADESH

A. Serologic Surveys, February 1970

Locality	Kalapara (pop. 1400)			Balihar (pop. 5300)		
	Number		Percent Positive	Number		Percent Positive
	Exam.	Posit.		Exam.	Posit.	
Age:						
0-1	22	5	22.7	32	0	0
2-4	93	29	31.2	62	0	0
5-9	303	145	47.9	28	3	10.7
10-19	243	143	58.9	35	10	28.6
20-29	121	83	69.6	14	9	64.3
30-39	113	84	74.3	15	11	73.3
40+	100	77	77.0	19	15	78.9
Total	995	566	56.9	205	45	21.9

B. Surveillance Information

1. Active Case Detection in Balihar

Year	Number		% Pos.	% falciparum	A.B.E.R.	A.P.I.
	Slide	Positive				
1964	141	39	27.7	61.5	10.1	2.8
1965	130	20	15.4	10.0	9.3	1.4
1966	58	2	3.4	-	4.1	0.1
1967	375	2	0.5	-	26.8	0.1
1968	104	1	0.9	-	7.4	0.07
1969	122	0	-	-	8.7	-

2. Mass Blood Survey, Kalapara

Year	Number		Percent Positive
	Slides	Positive	
1968	1315	124	9.4

Figure 4

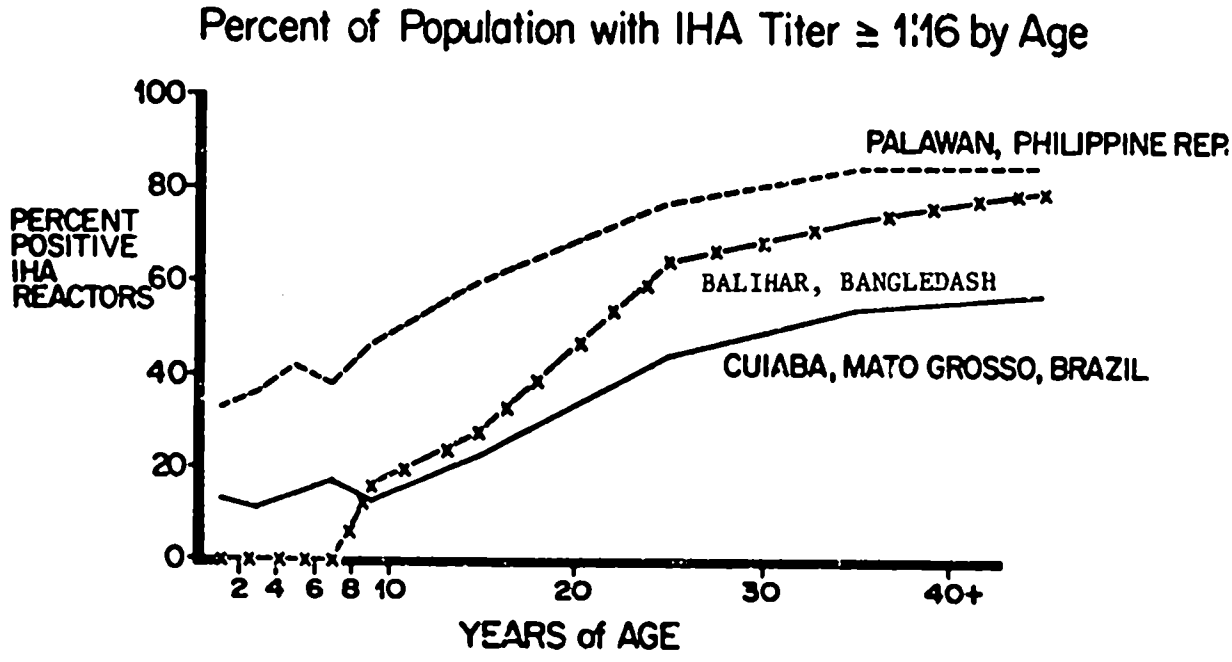

Percent of Population with IHA Titer ≥ 1:16 by Age
PERCENT POSITIVE IHA REACTORS
100
80
60
40
20
0
PALAWAN, PHILIPPINE REP.
BALIHAR, BANGLEDASH
CUIABA, MATO GROSSO, BRAZIL
2 4 6 8 10 20 30 40+
YEARS of AGE

TABLE 17

Brazil: Cuiaba, Mato Grosso

Age	Sex	Number Examined	Positive[1] No.	Positive[1] (%)	Mean Titer[2]	Positive Blood Slides Number	Positive Blood Slides (%)
0-4	M	55	7	12.7	1:43	-	-
	F	60	6	10.0	1:72	2	3.3
	TOTAL	115	13	11.3	1:55	2	1.7
5-14	M	170	32	18.8	1:46	-	-
	F	178	30	16.9	1:64	1	0.6
	TOTAL	348	62	17.8	1:54	1	0.3
≥15	M	139	79	56.8	1:175	5	3.6
	F	161	63	39.1	1:94	1	0.6
	TOTAL	300	142	47.3	1:133	6	2.0
All Ages		763	217	28.4	1:101	9	1.2

[1] IHA titer ≥1:16
[2] Of serologic positive specimens only

TABLE 18

BANGLADESH: BALIHAR

Age	Sex	Number Examined	Positive[1] No.	Positive[1] (%)	Mean Titer[2]	Positive Blood Slides Number	Positive Blood Slides (%)
0-4	M	47	-	-	-	-	-
	F	47	-	-	-	-	-
	TOTAL	94	-	-	-	-	-
5-14	M	23	4	17.4	1:23	-	-
	F	28	3	10.7	1:40	-	-
	TOTAL	51	7	13.7	1:29	-	-
≥15	M	35	25	71.4	1:99	-	-
	F	25	16	64.0	1:83	-	-
	TOTAL	60	41	68.3	1:92	-	-
All Ages		205	48	23.4	1:78	-	-

[1] IHA titer ≥1:16
[2] Of serologic positive specimens only

TABLE 19

Philippines: Palawan

Age	Sex	Number Examined	Positive[1] No.	Positive[1] (%)	Mean Titer[2]	Positive Blood Slides Number	Positive Blood Slides (%)
0-4	M	106	41	38.7	1:65	6	5.7
	F	100	39	39.0	1:130	6	6.0
	TOTAL	206	80	38.8	1:91	12	5.8
5-14	M	284	140	49.3	1:104	26	9.2
	F	288	134	46.5	1:126	15	5.2
	TOTAL	572	274	47.9	1:114	41	7.2
≥15	M	410	360	87.8	1:471	23	5.6
	F	356	254	71.3	1:340	14	3.9
	TOTAL	766	614	80.2	1:410	37	4.8
All Ages		1544	968	62.7	1:257	90	5.8

[1] IHA titer ≥1:16
[2] Of serologic positive specimens only

also compare the parasitologic and serologic data of the three surveys by age and sex. The risk of exposure to malaria appears to be or have been greater for adult males than for adult females in these areas.

It was possible to obtain information with the IHA method about the malaria experience of the population in Balihar although none of the blood slides contained Plasmodium parasites. Similarly, Buck et al., 1970, were able to determine the absence or presence of malaria transmission in four localities in Afghanistan during a survey which required only relatively few serum samples to be examined by the IHA method.*

The relatively long persistence of antibodies to malaria detected with the IHA test enables a single survey to determine the population profile which reflects the present risk of exposure and also the previous malaria experience. In comparison with parasitologic methods, the serologic technique requires that only a small sample of the population be examined. This is especially important when the IHA test is used as an independent means to estimate the effect of antimalaria measures and to assess the adequacy of the malaria vigilance system.

The serologic method may, therefore, be a valuable adjunct to the standard surveillance methods, especially in areas with a low degree of transmission and in areas where transmission has reportedly been eliminated.

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*Buck, A. A., Anderson, R. I., Kawaha, K., Sasaki, T. T., Amin, F. M., and Abrahams, I. W. 1970. Health and Disease in Rural Afghanistan. Johns Hopkins University, Baltimore, Maryland, pp. 161-166.

b. IFA test

1) Antigen studies

a) Preparation of antigen

Thin smears of parasitized whole blood were used as antigen in the malaria IFA when the program was initiated in our laboratory. With such preparations, organisms were often present in small numbers in any given area. For proper evaluation of test reactions, rather prolonged searches were often required to locate organisms. Infected blood for antigen had to be drawn before the donor had produced significant amounts of antibody. Since antibodies were present, it was said that an in vivo reaction of antibody with the organisms would occur and would render the material unsuitable for antigen. There were no reports of thick smear preparations of antigen. When parasitized cells are washed free of soluble serum components soon after being drawn from the donor, the donor's antibody level is of no consequence in antigen preparation. For instance, antigen prepared from donors with antibody titers of 1:1024 is satisfactory for use in the test. With this method the relatively large amounts of serum globulins present in whole-blood antigens, including antibodies, are eliminated. By this simple washing procedure, thick smear antigens are prepared for use in the IFA test.

This method of antigen preparation has several advantages. Removing the donor's gamma globulin decreases nonspecific staining of the background. The number of organisms per microscopic field is increased. This increase makes reading of the test more rapid and less laborious and insures that all life cycle stages that are reactive in the test will appear in each field.

b) Evaluation of thick smear antigen

When the washed-cell thick smear was used to test sera from persons proven to have malaria, reactions at dilutions of 1:16 or greater were obtained in more than 95 percent of the sera tested. All sera from persons with no history of exposure to malaria, including 26 syphilitic teenage Americans, were negative at 1:16 and above. A battery of sera were tested from 184 persons who had no exposure to malaria (128 American Indian school children, 105 of whom had one or more intestinal parasites, plus 56 Eskimos from Alaska) and from 49 persons with slide proven malaria (Table 20). These sera were assorted by a system of random numbers and coded. Tests were performed blind. This battery was tested with antigens of P. falciparum, P. vivax, and P. brasilianum, the latter being considered as serologically identical with P. malariae. Reactions at 1:16 occurred with only one serum of the unexposed group and with only P. falciparum antigen, for a specificity of 99.5 percent. Of the 49 sera from known cases of malaria, 46 reacted at 1:16 or greater with at least one of the antigens (several reacted only with the antigen homologous to the infecting species) for a sensitivity of 94 percent. To study the reproducibility, we split the 49 positive sera into duplicates, assorted, and coded them (Table 21). Tests with the three antigens gave a reproducibility of 97 percent within plus or minus one fourfold dilution. Reproducibility of titers was also determined with two sera tested 40 or more times with the antigens mentioned above and more than 10 times with P. fieldi antigen (Table 22). Reproducibility was 99 percent within the plus or minus one fourfold dilution limit. Also in this study, 46 sera from P. vivax malaria and 15 from P. falciparum were tested with P. vivax and P. falciparum antigens (Table 23). There was no difference in titer between the two antigens in nine cases, and the titer was greater at a 64-fold level with the heterologous antigen in only one case. Since

TABLE 20

Number and percent of reactors to three plasmodium antigens among 184 sera from individuals not exposed to malaria and 49 sera from individuals with a positive slide diagnosis.

Sera Group	Titers	Washed-cell thick-smear antigens					
		P. falciparum		*P. vivax*		*P. brasilianum*	
		No.	%	No.	%	No.	%
Donors not exposed to malaria	Negative	157	85.3	180	97.8	183	99.5
	1:4	26	14.2	4	2.2	1	0.5
	1:16	1	0.5	0	0.0	0	0.0
Donors with positive slide diag.*	Negative	6	12.2	4	8.2	9	18.4
	1:4	10	20.4	4	8.2	3	6.1
	1:16	33	67.4	41	83.6	37	75.5

*All sera in this group had previously had titers of at least 1:16 with one of the three antigens. On this second titration, two of these sera had negative reactions with all three antigens.

TABLE 21

Day-to-day reproducibility of IFA titers based on two titrations of 49 slide-positive sera with three antigens.

Reproducibility*	Antigen			Total
	P. falciparum	*P. vivax*	*P. brasilianum*	
Number of sera with exact agreement on both titers	19 (39%)	23 (47%)	16 (33%)	58 (40%)
Number of sera with a 4-fold difference between titers	22 (45%)	23 (47%)	21 (43%)	66 (45%)
Number of sera with a 16-fold difference between titers	6 (12%)	3 (6%)	9 (18%)	18 (12%)
Number of sera with a 64-fold difference between titers	2 (4%)		3 (6%)	5 (3%)
Total sera tested	49	49	49	147 (100%)

*Differences in reproducibility among the 3 antigens are not statistically significant by a chi-square test.

TABLE 22

Distribution of titers for two antisera with four antigens in the indirect fluorescent antibody (IFA) test for malaria

Reciprocal Titer	Plasmodium Antigens							
	P. vivax		*P. falciparum*		*P. brasilianum****		*P. fieldi*	
	E.T.*	MOR.**	E.T.	MOR.	E.T.	MOR	E.T.	MOR.
Negative				6		4		2
4				14		9	2	3
16	16	9	22	27		25	10	8
64	29	30	28	2				
256		1			6			
1024					34			
4096					2			
Total replicates	45	40	50	49	42	38	12	13

*Plasmodium malariae demonstrated in circulating blood. Mother of an infant with congenital malaria.[4]

**District medical officer, Sepik, New Guinea. Reported Plasmodium vivax infection with treatment one year prior to sampling.

***P. brasilianum antigen was used as an equivalent to P. malariae.

TABLE 23

Comparison of infecting species of plasmodium with difference in antibody titer obtained with homologous and heterologous antigen in vivax and falciparum malaria

	Number of sera	Number vivax	Number falciparum
No difference in titer	9	7	2
Homologous titer greater:			
4-fold	12	7	5
16-fold	11	8	3
64-fold	12	8	4
256-fold	9	9	0
1024-fold	4	4	0
Heterologous titer greater:			
4-fold	3	2	1
16-fold	0	0	0
64-fold	1	1	0
Totals	61	46	15

All donors were military returnees from Vietnam. Many gave history of recurrent attacks of malaria. This was true of the case giving a heterologous titer 64-fold greater than that with the infecting plasmodium at the time the specimen tested was drawn.

these sera were from individuals who were exposed to both species of malaria, the fact that 94 percent of the titers were greatest with the antigen homologous to the infecting species indicates that the test could be used to determine the infecting species.

c) Schizont antigens

It has been recognized for some time that more mature stages of Plasmodium have greater reactivity than do the less mature forms. This observation makes it imperative, when comparisons of Plasmodium antigens are made, that similar stages of antigen be employed. Because of the greater reactivity of schizont antigens, these antigens may differ in specificity and other test parameters from the trophozoite antigen. A pilot study confirmed that the schizont antigen will result in greater reactivity than the trophozoite antigen. Another finding was that the quality of conjugate also influences the reactivity.

For the final evaluation of schizont antigens, a total of 320 positive and negative sera were tested. The test with the new antigen and new conjugate is very sensitive and new criteria for determination of positive and negative reactions have to be used. When a dilution of 1:64 is used for a positive reaction, the test has a sensitivity of 96.5 percent and a specificity of 94 percent.

d) Multi-species antigen

Testing each serum with antigens of different malaria species on four different slides is both time consuming and requires relatively large amounts of reagents. Two lots of antigen consisting of P. vivax, P. falciparum, and P. brasilianum (P. malariae simian equivalent) were made by combining the three bloods on one slide in a single drop. Test results indicate that titers obtained with the multi-species antigen can be used to screen and titrate large numbers of sera in a fraction of the time previously required.

2) Speciation studies

To further test the possibility of identifying the infecting species, sera from 159 malaria patients (120 P. vivax and 39 P. falciparum slide proven cases) were tested with P. vivax and P. falciparum antigens. In this group, when a fourfold titer difference was considered significant (since comparison was on a within-test basis), 77 percent were correctly diagnosed as to species, 4 percent were misdiagnosed, and because of lack of titer difference, 19 percent could not be determined. Since these were natural infections, exposure to both species may have occurred in many cases, and could account for some of the misdiagnosed and undetermined cases. Serial specimens from 79 servicemen diagnosed by morphological examination of blood slides as P. vivax or P. falciparum malaria were also tested. Species determination on sera taken during the first two weeks of the clinical disease were successful in 81 percent of the cases. Sera taken 15 to 60 days after the day of onset of clinical symptoms reacted at titers at least fourfold greater with the antigen homologous to the infecting species in 96 percent of the cases. Efficiency of speciation by the IFA test decreased rapidly within 6 months, when infecting species could be determined in only 40 percent of the cases, but with misdiagnosis still only 4 percent; many sera from the 56 percent undetermined cases gave no reaction.

3) Antibody patterns

a) Multiple serum specimens from 24 persons with induced infections of either P. vivax or P. falciparum were tested to determine how early in infection the IFA schizont antigens detect malarial antibody. The 109 sera tested included preinfection samples and specimens taken up to 2 weeks after onset of parasitemia. The results with these sera indicated that initial antibody could be detected at approximately the time of onset of parasitemia.

b) Sera were also tested from 58 patients who had been admitted to the hospital for treatment of malaria. The patients were servicemen who had

acquired their infections in Vietnam. The diagnosis was based on identification of Plasmodium parasites in peripheral blood smears. The sera were obtained on the day of admission. Only six sera were negative with the IFA test. Two to 4 weeks later a second serum specimen was obtained and all but one of these patients then had a titer of 1:16 or higher. The one serologically negative patient showed scanty parasites in the blood only once, and he was promptly treated. He recovered without having developed a detectable antibody level. The geometric mean titer of the group was 1:184 on the day of admission, 1:178 2 to 4 weeks later, 1:9 6 months later, and 1:8 after 12 months.

c) It has been suggested that relapses or reinfections result in increased antibody titers. In 47 cases of servicemen with clinical malaria, 23 gave a history of past malarial episodes and 24 did not. When the IFA test was performed on sera drawn in the first 2 weeks, 2 to 4 weeks, 4 to 13 weeks, 26 to 38 weeks, and 39 to 52 weeks after the onset of illness, no differences in antibody titer patterns between the two groups were noted.

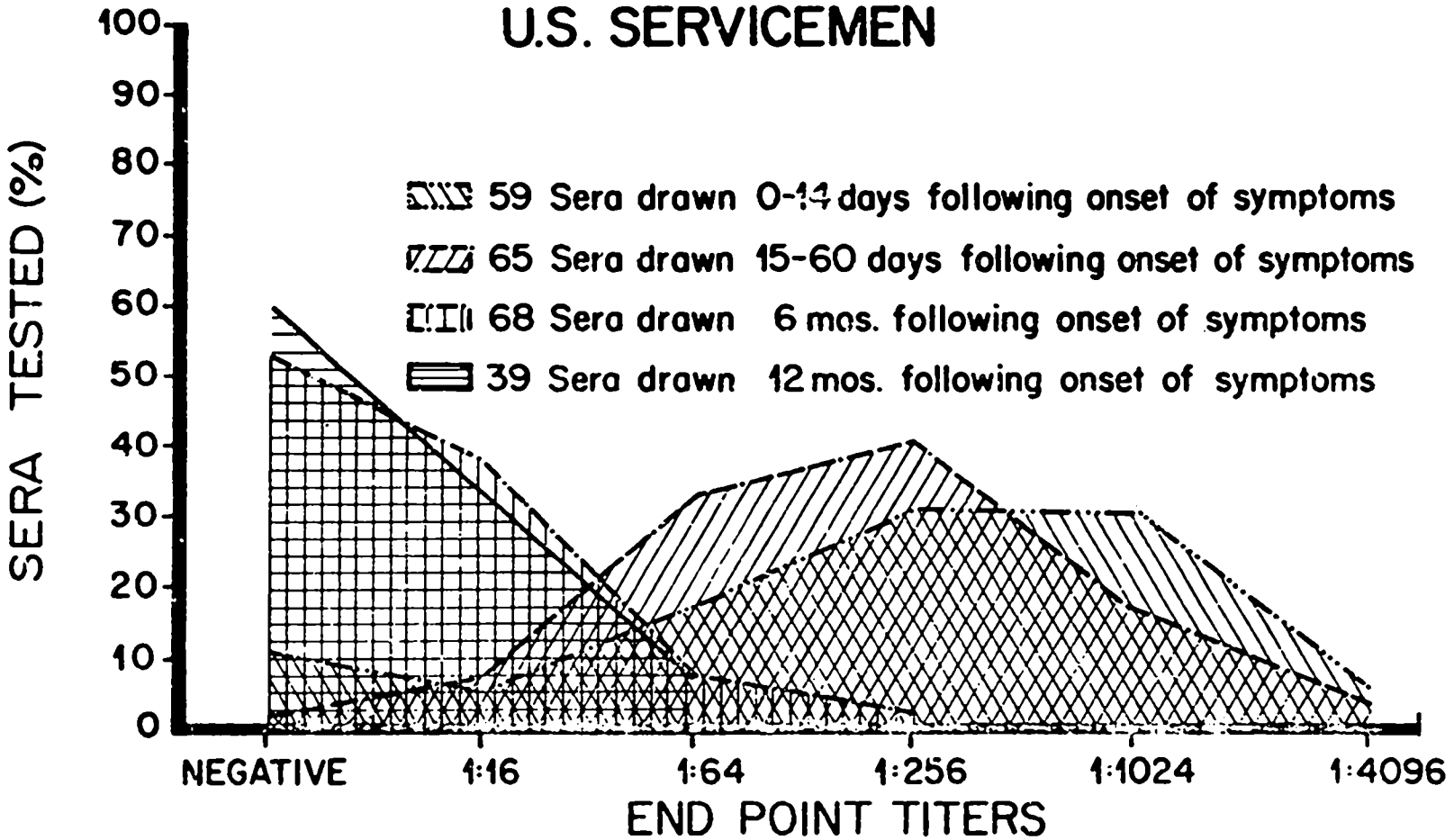
It was also of interest to determine the antibody patterns in such cases after radical cure. This study revealed that antibody peaked in this group within 60 days, and a titer with homologous antigen of 1:256 or greater may suggest very recent infection (Figure 5).

4) Use of immunoglobulin specific conjugates

a) To evaluate a CDC-IgM conjugate, we tested sera from 22 patients who had been infected with malaria. This included 16 acute sera and six sera from individuals who had been treated 6 months previously. The 16 acute specimens gave positive IgM reactions whereas the other six specimens were negative.

FIGURE 5

MALARIA IFA TEST
ANTIBODY TITER PATTERNS WITH HOMOLOGOUS ANTIGENS IN U.S. SERVICEMEN

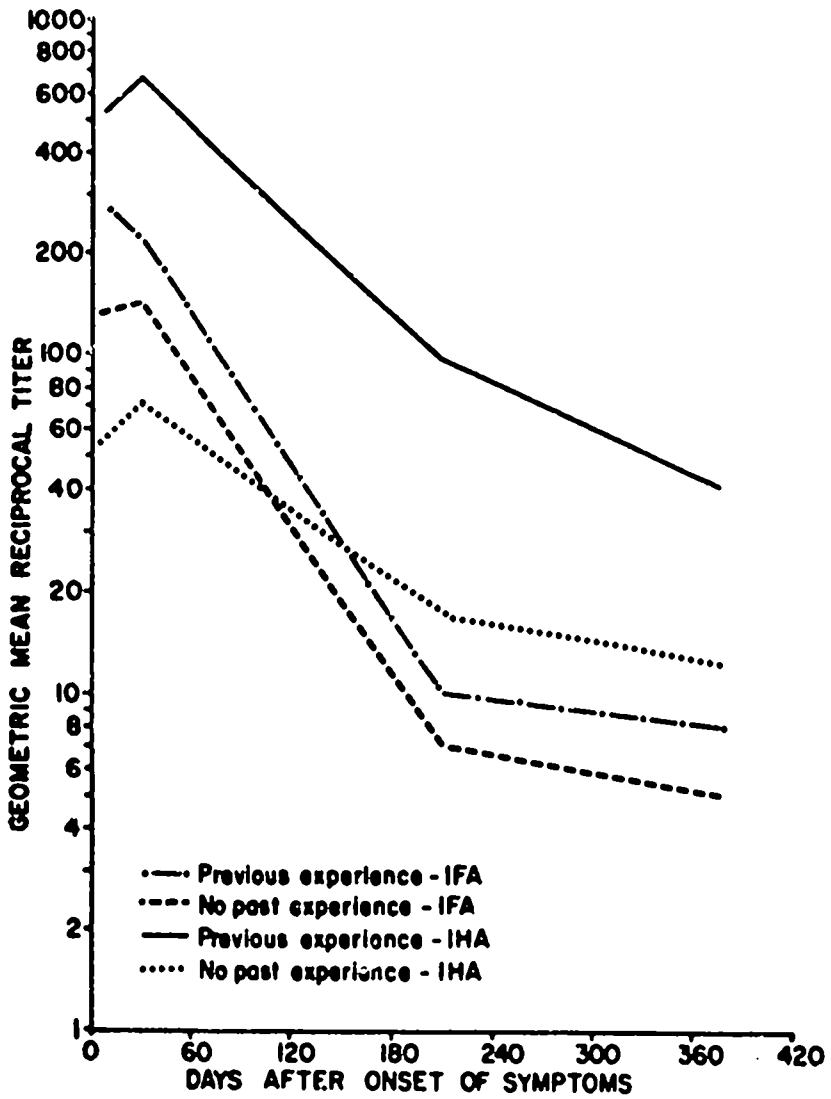


b) A total of 258 filter paper blood specimens collected in October 1971 in Nigeria were tested by IFA; 257 specimens were positive at 1:64 or greater when tested with the multispecies antigen. IgM conjugate was used to test an additional 101 specimens, 50 of which had an IgM reaction at 1:32.

5) Comparisons with other tests

The IFA and IHA test results were compared on sera drawn from 68 servicemen who experienced P. vivax malaria after return to the United States. Blood was drawn on admission, after 2 weeks, at 2 to 8 weeks, and at 7 and 12 months after radical cure (Figure 6). With the two procedures, it was found that in the first 2 weeks IFA results were positive in 90 percent of the cases and IHA in 83 percent of them. For the 2- to 8-week interval, the corresponding percentages were 98 and 92 percent; at 7 months, 45 percent and 84 percent; and after 12 months, 32 percent and 77 percent. The IFA test seems to detect antibodies earlier in the course of the disease, and the IHA test appears to detect antibodies after radical cure for a much longer period than the IFA test. When the patients were divided into those with past and no past history of malaria and test results were compared on the basis of geometric mean reciprocal titer (GMRT), the IFA test was found to be best for detecting malaria in those experiencing their first attack and for detection of exposure in the past. We also compared the IFA and IHA test results for about 400 sera from individuals involved in an outbreak of P. vivax malaria among heroin addicts who shared syringes and needles. Forty-six sera were psoitive by IFA and 68 by IHA tests in this group. In 53 slide positive cases, four were found negative by both tests, and 42 were positive by both tests; three were negative by the IFA test and positive by the IHA, and the reverse was true in two cases.

Figure 6


1000
800
600
400
200
100
80
60
40
20
10
8
6
4
2
1
GEOMETRIC MEAN RECIPROCAL TITER
0
60
120
180
240
300
360
420
DAYS AFTER ONSET OF SYMPTOMS
Previous experience - IFA
No past experience - IFA
Previous experience - IHA
No past experience - IHA

6) Transfusion malaria

Transfusion-induced malaria indicates that occult malaria may exist and be undetectable by slide examination. The identity of the responsible donor in cases of multiple transfusions often can only be inferred from history. The IFA test furnishes a more reliable tool. In nine cases of transfusion-induced malaria where the donors could be tested, all responsible donors could be identified by IFA tests, and the infecting species was determined serologically in eight of the cases (Table 24). In the one case not resolved (#10), not all the donors were available for testing. Histories that were taken separately and compared with IFA test results confirmed these findings. Results of this study suggest that the IFA test could be used to detect blood donors who might have malaria infections.

3. Research Design

a. The IHA test

As indicated above, it has been necessary to develop a method to stabilize the red cell carrier. Improving the production and evaluation of the antigen for the IHA test is still a problem and will require additional work.

Although performing the IHA test technique is relatively simple, use of automation will make it possible to test more specimens and may also provide better standardization of the test procedures.

A major advantage of the IHA test is the large number of specimens that can be processed. This laboratory advantage has to be matched with an equally rapid data handling and analysis method. Delay of data analysis has to be minimal so that the information can be of maximum use for the malaria programs.

A computer data analysis system has been developed which can handle the information from a single study. However, longitudinal studies and use of the IHA test in the

TABLE 24

IFA REACTIONS OF SERUM SAMPLES FROM BLOOD DONORS INVOLVED IN 10 CASES OF TRANSFUSION-INDUCED MALARIA

Case No.	Infecting species	No. of donors	No. tested by IFA	No. positive by IFA	IFA Titers		
					vivax	falciparum	malariae
1	P. falciparum	2	2	1	1:16	1:256	Neg.
2	P. malariae	2	2	1	1:16	1:16	1:256
3	P. falciparum	4	4	1	1:64	1:1024	1:256
4	P. malariae	12	2	1	1:64	1:16	1:1024
5	P. falciparum	7	3	1	1:64	1:256	1:256
6	P. malariae	56	9	1	1:64	1:256	1:4096
7	P. falciparum	6	6	1	1:64	1:256	1:64
8	P. falciparum	13	13	1	1:16	1:256	1:16
9	P. falciparum	1	1	1	Neg.	1:256	Neg.
10	P. vivax	7	5	0			

surveillance of malaria will require systems which can analyze and interpret repeated observations on the same individuals and/or populations.

A few pilot field studies have already been carried out to test the operational use of serologic methods in malaria programs, and the results have been very promising. However, progress in this important aspect in the development of the techniques has been difficult because we are almost entirely dependent on our collaborators abroad to design the studies, to determine the specimen collection method, to obtain the related malariometric data, and to provide us with the epidemiologic information necessary for interpreting the results.

It is therefore important for our program personnel to be directly involved in the field studies. They must be able to study and assess the factors which can influence the test results. These include not only the various aspects of the laboratory procedures in Atlanta but also the methods of selecting the population sampled, the specimen collection and preservation methods, the accuracy and completeness of recorded data and the blood slide examination results, and important variables like age, sex, residence, population movements, occupation, and history of malaria or other diseases. It is also important to know the completeness and validity of the traditional parameters used to measure malaria in the population groups studied. This requires detailed knowledge about malaria in the area. Lack of such direct knowledge has often considerably limited our ability to interpret the test results. More direct participation in the studies will also enable us to indicate to the participating Malaria Eradication Programs how to use the serologic method to meet their specific needs.

b. The IFA test

Development of a thick smear antigen has made use of the IFA test more practical. Studies also disclosed that schizont antigens were more reactive than trophozoite antigens, and the quality of the conjugate also influences the reactivity.

The multi-species antigen makes use of the IFA test more practical, especially when detection of antibodies to more than one Plasmodium species is desirable. Detection of antibodies to several species is now no more time consuming than detecting antibodies to a single species, although mono-specific antigens still have to be used for species identification.

E. Dissemination and Utilization of Research Results

Our studies demonstrate how seroepidemiologic methods can be applied to programs within a given country. The staff of each program collaborating in our studies participates in interpreting the results. This arrangement enables the directors of the malaria programs to put the information to immediate use. Thus, research results can be directly applied to existing problems. Our collaboration with the program directors in each country enables us to demonstrate new approaches and show how the serologic technique can best be used for malaria surveillance within their operational system.

Studies have been and still are conducted with international organizations (e.g., a 4-year study with the World Health Organization in Nigeria); other groups at CDC (Malaria Program, CAMRS); USAID/CDC advisors and local personnel of USAID supported Malaria Eradication Programs in Bangladesh, Nepal, Ethiopia, the Philippines, Brazil, El Salvador, and Haiti; NAMRU-2 and NAMRU-3 (U.S. Naval Medical Research Unit) for studies in Ethiopia and Indonesia; and other research workers in the United States and abroad.

Many laboratories have now adopted the thick smear antigen for use in the IFA test for malaria. The serologic methods are used in the United States to detect donors of malaria-infected blood, in the investigation of drug-associated outbreaks of malaria, and in other epidemiologic investigations of malaria. There are several examples that results of our research projects have been useful in lesser developed countries (LDC's). The Mato Grosso area in Brazil was thought to be highly malarious and was selected for an extensive insecticide trial program. Two persons from CAMRS worked in the area for 1 month and 1,500 blood slides were examined. At the same time, they collected blood samples on filter paper, and the samples were processed in 3 days at CDC. The study results revealed little malaria

in the area, and plans for the spraying program were discontinued. The validity of the serologic survey was confirmed by several blood slide surveys. The initial judgment that this area in the Mato Grosso was highly endemic was based on data from the routine malaria surveillance system which proved to be inadequate. The speed with which results can be obtained by the serologic method is important.

Dr. Alfred Buck of the World Health Organization conducted serologic surveys in several villages in Afghanistan. The results of the IHA studies indicated no recent malaria transmission in the village of Saidabad but active transmission in two other localities. The serologic population profile in the village of Bulla Quchi in the Kunduz area near the Oxus River in the northeastern part of Afghanistan indicated some recent transmission although malaria had reportedly been eradicated and no blood slides had been found to be positive. Finding malaria antibody in the 1- to 4-year age group in the Oxus River region in Afghanistan was the signal that transmission was taking place. One year after we made our studies, the first serious outbreak occurred; subsequently, extensive antimalaria measures had to be reinstituted.

Similarly, the interruption of transmission in Balihar, Bangladesh, was demonstrated by a single survey. No antibodies were found in persons under 8 years of age. The success of the Malaria Eradication Program in Balihar was confirmed by the Active Case Detection information.

Reports of the research activities are prepared quarterly and annually; they are distributed to AID/W and to malaria research groups at CDC and elsewhere. Reports are sent to our collaborators in the laboratory work, in the field studies, to WHO, and to PAHO. Lectures on the seroepidemiology of malaria are given at universities inside and outside of the United States. Our results are published in scientific journals and in the WHO-MAL series. Scientific papers containing the details of the research results are presented at meetings of professional societies and at seminars.

The research program is discussed with and explained to the many domestic and foreign visitors to the Parasitology Branch, CDC, who are involved in malaria programs and in malaria research. Personal contacts with persons in WHO, PAHO, and the country Malaria Eradication Programs also provide us with many opportunities to discuss how results of this research program can be used in the management of Malaria Eradication Programs.

F. Expenditures and Obligations and Contractor Resources

Object	FY-1968	FY-1969	FY-1970	FY-1971	FY-1972
11	14,092.80	30,714.48	50,648.64	71,144.60	73,902.79
12	2,052.16	2,320.04	3,605.63	5,657.24	5,948.24
21	11.95	845.30	1,794.58	3,080.53	3,398.84
22	23.79	.00	.00	.00	.00
23	.00	.00	300.00	400.00	400.00
24	200.00	.00	.00	.00	.00
25	252.00	882.40	775.00	.00	626.18
26	5,893.13	13,162.78	12,876.15	5,341.63	8,223.95
31	3,745.00	7,075.00	.00	6,833.00	.00
Direct	26,270.83	55,000.00	70,000.00	92,457.00	92,500.00
+ CDC indirect	5,254.17	11,000.00	14,000.00	18,491.00	18,500.00
TOTAL	31,525.00	66,000.00	84,000.00	110,948.00	111,000.00

10/19/72

C. Appendix

1. Bibliography of Project Results

Sulzer, A.J. and Wilson, M., 1967. The use of thick-smear antigen slides in the malaria indirect fluorescent antibody test. J. Parasit., 53:1110-1111.

Rogers, W.A., Jr., Fried, J.A., and Kagan, I.G., 1968. A modified indirect microhemagglutination test for malaria. Am. J. Trop. Med. Hyg., 17:804-809.

Kagan, I.G. and Rogers, W.A., Jr., 1968. Evaluation of the indirect microhaemagglutination test for malaria. WHO ME/SGPM/WP/68.11.

Kagan, I.G., Mathews, H.M., and Sulzer, A.J., 1969. The serology of malaria: Recent applications. Bull. N.Y. Acad. Med., 45: 1027-1042.

Kagan, I.G., Mathews, H.M., Rogers, W.A., Jr., and Fried, J.A. 1969. Seroepidemiological studies by indirect haemagglutination tests for malaria. Military recruit collections from Argentina, Brazil, Colombia, and the United States of America. Bull. Wld. Hlth. Org., 41:825-841.

Harvey, B., Remington, J.S., and Sulzer, A.J., 1969. IgM malaria antibodies in a case of congenital malaria in the United States. The Lancet, Feb. 1969, 333-335.

Sulzer, A.J., Wilson, M., and Hall, E.C., 1969. Indirect fluorescent antibody tests for parasitic diseases. V. An evaluation of a thick-smear antigen in the IFA test for malaria antibodies. Am. J. Trop. Med. Hyg., 18:199-205.

Mathews, H.M., Fisher, G.U., and Kagan, I.G., 1970. Persistence of malaria antibody in Tobago, West Indies, following eradication as measured by the indirect hemagglutination test. Am. J. Trop. Med. Hyg., 19:851-855.

Mathews, H.M., Fried, J.A., and Kagan, I.G., 1970. A seroepidemiologic study of malaria in the Republic of the Philippines by the indirect hemagglutination test. Am. J. Epid., 92:376-381.

Fisher, G.U., Sulzer, A.J., Wilson, M., and Runcik, K., 1970. Fluorescent antibody patterns in naturally acquired vivax malaria. Am. J. Trop. Med. Hyg., 19:209-214.

Wilson, M., Sulzer, A.J. and Runcik, K., 1970. Malaria antibody patterns as determined by the IFA test in U.S. servicemen after chemotherapy. Am. J. Trop. Med. Hyg., 19:401-404.

Fisher, G.U., Gordon, M.P., Lobel, H.O., and Runcik, K., 1970. Malaria in soldiers returning from Vietnam. Epidemiologic therapeutic and clinical studies. Am. J. Trop. Med. Hyg. 19:27-39.

Wilson, M., Sulzer, A.J., Rogers, W.A., Fried, J.A., and Mathews, H.M., 1971. Comparison of the indirect fluorescent antibody and indirect hemagglutination tests for malarial antibody. Am. J. Trop. Med. Hyg., 20:6-9.

Gleason, N.M., Wilson, M., Sulzer, A.J., and Runcik, K., 1971. Agreement between microscopical diagnosis and indirect fluorescent antibody tests in Plasmodium vivax and Plasmodium falciparum infections. Am. J. Trop. Med. Hyg., 20:10-13.

Sulzer, A.J. and Wilson, M., 1971. The indirect fluorescent antibody test for detection of occult malaria in blood donors. Bull. Wld. Hlth. 45:375-379.

Kagan, I.G., 1971. Malaria: seroepidemiology and serologic diagnosis Experimental Parasitol, 31: 126-135.

Sulzer, A.J., and Wilson, M., 1971. The fluorescent antibody test for malaria. CRC Crit. Rev. Clin. Lab. Sci. 2:601-619.

Sulzer, A.J., Wilson, M., and Turner, A., 1972. A multispecies malaria antigen for use in the indirect fluorescent antibody test. Trans. Roy. Soc. Trop. Med. Hyg. (in press).

Ludford, C.G., Sulzer, A.J., Wilson, M., and Hall, W.T.K., 1972. Antigenic cross-reactions between Babesia argentina and Plasmodium vivax and Plasmodium falciparum. Exp. parasit. (in press).

Kagan, I.G., 1972. Evaluation of the indirect hemagglutination test as an epidemiologic technique for malaria. Am. J. Trop. Med. & Hyg. 21: 683-689.

Lobel, H.O., Kagan, I.G., and Mathews, H.M., 1972. The indirect microhemagglutination test for malaria and its use in antimalaria programs (in manuscript).

Farshy, D.C., and Kagan, I.G., 1972. Use of stable sensitized cells in the microhemagglutination test for malaria. Am. J. Trop. Med. & Hyg. (in press).

Farshy, D.C., and Kagan, I.G., 1972. Improved microhemagglutination test for malaria using stable sensitized cells (in manuscript).

2. Other Reports and Uses of Research Project

Sulzer, A.J. and Wilson, M., 1967. A washed cell thick smear antigen for the malaria IFA test. Am. Soc. Trop. Med. & Hyg.

Kagan, I.G. and Rogers, W.A., 1968. Evaluation of the indirect microhemagglutination test for malaria. Eighth Intern. Congr. of Trop. Med. & Malaria. Teheran, Iran.

Sulzer, A.J., Wilson, M., and Hall, E.C., 1968. Evaluation of a thick smear antigen in the indirect fluorescent antibody test for malaria. Am. Soc. Trop. Med. & Hyg.

Wilson, M. and Sulzer, A.J., 1968. Rise and fall of titers in the indirect fluorescent antibody test for malaria. Am. Soc. Trop. Med. & Hyg.

Kagan, I.G., Mathews, H. and Sulzer, A.J., 1969. The serology of malaria. Symposium on malaria. Tropical Disease Center, New York City.

Sulzer, A.J. and Wilson, M., 1969. Serological relationships of Plasmodium species in malaria IFA tests. Am. Soc. Trop. Med. & Hyg.

Sulzer, A.J. and Wilson, M., 1969. A long term, high level peripheral parasitemia due to Plasmodium brasilianum in a spider monkey, Ateles geoffryi. Am. Soc. Parasit.

Mathews, H.M., Fisher, G.U., Kagan, I.G., and Fried, J.A. 1969. Persistence of malaria antibodies following eradication in an endemic area. Indirect hemagglutination studies in Tobago. Soc. Trop. Med. & Hyg.

Wilson, M., Sulzer, A.J., Mathews, H.M., and Fried, J.A., 1969. Comparison of the indirect fluorescent antibody and indirect hemagglutination tests for malarial antibody. Am. Soc. Trop. Med. & Hyg.

Kagan, I.G. and Mathews, H.M., 1970. Application of the indirect hemagglutination test as an epidemiologic technique for malaria. Inter-American Malaria Research Symposium. San Salvador, El Salvador.

Kagan, I.G., 1970. Immunodiagnosis of malaria and other parasitic diseases. Symposium at the University of Ohio, Columbus, Ohio.

Lobel, H.O., Mathews, H.M. and Kagan, I.G., 1971. The persistence of antibodies to malaria detected with the indirect hemagglutination test and application of the test in malaria surveillance. Am. Soc. Trop. Med. & Hyg.

Kagan, I.G., 1971. Evaluation of the indirect hemagglutination test as an epidemiologic technique for malaria. Inter-American Malaria Research Symposium. San Salvador, El Salvador.

Ludford, C.G., Sulzer, A.J. and Wilson, M., 1971. Antigenic cross reactions between Babesia Argentina, Plasmodium vivax, and Plasmodium falciparum. Am. Soc. Trop. Med. & Hyg.

Mathews, H.M. and Fried, J.A., 1971. Evaluation of a filter paper method of blood collection for use in malaria serology. Am. Soc. Trop. Med. & Hyg.

Geoffery, G.M., Warren, McW., Colling, W.E. and Mathews, H.M. 1971. Longitudinal seroepidemiologic studies on malaria in the Mato Grosso area of Brazil. Am. Soc. Trop. Med. & Hyg.

Four year malaria study in Nigeria by World Health Organization, Malaria Eradication Division. ?

Investigations in the United States of narcotics associated malaria, episodes of transfusion malaria, and of shipboard malaria. ?

IFA thick film procedure has been adopted by the laboratories of the London School of Tropical Medicine; the Nuffield Institute, London; the University of Ghana, Accra; the Fifth U. S. Army, Ft. Sam Houston, Texas; the University of Nymegen, Holland; the University of Grenoble, France; and the Institute for Tropical Medicine in São Paulo, Brazil.

Comprehensive health survey in Afghanistan by A. A. Buck, et al.

Studies by NAMRU-2 in Indonesia and NAMRU-3 in Ethiopia.

U. S. Army study of malaria in the 82nd Airborne Division. ?

Cooperative studies with CAMRS in Brazil and El Salvador; the Nuffield Institute, London; the Laboratory for Parasitic Diseases, NIAID, NIH, Chamblee, Georgia; the Walter Reed Army Institute for Research, Washington, D. C.; the University of Grenoble, France; the University of Nymegen, Holland; the Swiss Tropical Institute, Basel; and the Malaria Eradication Programs in Haiti, Bangladesh, the Philippines, New Guinea, Ethiopia, and Nepal.

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15. Supplementary Notes

16. Abstracts

Microscopists in malaria programs examine millions of blood slides each year. In areas with a low incidence of infection, few positive blood films are found, and more sensitive epidemiologic tools are needed. Even in regions with a high incidence of malaria, where determination of spleen sizes could indicate the level of endemicity, a more specific method is required since splenomegaly is a nonspecific physical finding and may be due to causes other than malaria. Thus, the development of serologic methods to detect the presence of antibodies against malaria would provide an improved epidemiologic tool.

The main objective of the project was to develop simple, rapid, accurate methods for the serologic diagnosis of malaria for use in malaria programs. Diagnostic techniques based on malaria serology were known in principle, but were not developed for field use to meet the large scale case finding and investigational needs of malaria programs. The use of the indirect hemaggluti-nation (IHA), indirect fluorescent antibody (IFA), and complement fixation (CF) tests and other serologic techniques had to be examined.

Another objective was to produce antigens from monkey and human malarias and to evaluate the sensitivity and specificity of such antigens for the diagnosis of human malaria.

The third objective was to determine the operational use of serologic techniques for epidemiologic and diagnostic purposes.

17b. Identifiers/Open-Ended Terms

*Serology, *Antigens and antibodies, *Malarial fever

17c. COSATI Field/Group 614

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