AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
Batch 43

1. SUBJECT CLASSIFICATION
A. PRIMARY: TEMPORARY
B. SECONDARY:

2. TITLE AND SUBTITLE
Serologic diagnosis of malaria; annual report,1970

3. AUTHOR(S)
(101) HEW/PHS

4. DOCUMENT DATE
1970

5. NUMBER OF PAGES
19p.

6. ARC NUMBER
ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
PHS

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)
(Research summary)

9. ABSTRACT

(HEALTH R & D)

10. CONTROL NUMBER
PN-AAC-876

11. PRICE OF DOCUMENT

12. DESCRIPTORS

13. PROJECT NUMBER

14. CONTRACT NUMBER
PASA RA(HA)-5-68 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

PASA RA(HA)-5-68 Res.
PN-AAC-876

Partial Synopsis of the Work
Accomplished By
AID PASA Control No. RA (HM) 5-68

by

Irving G. Kagan, Ph.D.
Parasitology Branch
Laboratory Division
CENTER FOR DISEASE CONTROL
HSMHA, PHS, DHEW
Atlanta, Georgia 30333

In 1955 the Eighth World Health Assembly recommended that malaria be eradicated from the earth. By the end of 1969, malaria had been eradicated in areas affecting approximately 699 million people. An additional 703 million people are living in countries where eradication is in progress. Approximately 376 million people live in areas where malaria eradication has not been initiated (World Health Organization, 1970).

The malaria eradication program has not been equally effective in all parts of the world. Years after eradication programs were conducted in Ceylon, outbreaks of malaria reached epidemic proportions. In Central America, partly because of changing economic conditions and mass population shifts, eradication programs have faltered. There may be more malaria in some parts of Central America today than before eradication programs were initiated. Setbacks in eradication programs in many countries suggest a need for more efficient surveillance methods. Toward this end, serologic methods have been evaluated as epidemiologic tools.

Immunologic methods employed in malaria are indirect fluorescent antibody (IFA), agar gel diffusion, complement fixation, latex, and indirect hemagglutination (IHA) tests. In our laboratory, we have employed the IHA test for epidemiologic purposes and the IFA test for diagnosis. In this report the evaluation of the IHA test and some seroepidemiologic applications will be presented.

The program for the serologic diagnosis of malaria was initiated at a time when epidemiologic studies on malaria were based on blood and spleen surveys, detection of fever cases, and identification of malaria parasites in stained blood films. These techniques, however, have their limitations. 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.

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.

A practical serologic method for use in malaria programs must meet the following criteria: a) the test has to be simple to perform; b) the interpretation of results must be free of subjectivity; c) the test must be rapid; d) the cost must be minimal; e) the test results have to be sufficiently sensitive and specific; f) the

test must be capable of producing reliable results in various laboratories, and g) must lend itself readily to automation. The IHA test now appears to meet these criteria better than other available serologic methods such as the IFA test, the soluble antigen fluorescent antibody (SAFA) test and the CF test.

The results of our research suggest that the 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 were also conducted to investigate the operational potential of the IHA test.

Pilot scale studies indicate that the IHA test may have a wide range of applications in epidemiology: 1) to measure the level of malaria endemicity, 2) to verify the absence or presence of transmission in a population by testing a relatively small number of specimens, 3) to delineate malarious areas, 4) to detect seasonal changes of malaria transmission; 5) to investigate suspected reintroductions of malaria into consolidation or maintenance phase areas; and 6) to detect foci of malaria transmission. The test is especially useful in areas where the regular malaria surveillance network is limited, and it can be used as a screening mechanism to detect potential carriers of malaria. It can also be used to measure the effect of antimalarial activities on the transmission of malaria and to assess the efficacy of the malaria surveillance system.

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.

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.

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 gluteraldehyde. 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.

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 and intensity of antigenic stimulations and the age of the patient.

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 antimalaria campaigns, and to compare the levels of malaria transmission in different areas.

The use of filter paper for the collection of blood has enlarged the value and usefulness of seroepidemologic methods. 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.

The development and persistence of antibodies is an important variable to measure in seroepidemiologic slides. 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.

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 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. 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.

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. Negative titers in 99 percent of the U. S. recruits confirmed the absence of malaria in this country. In the Brazilian and Colombian recruits, a serologic prevalence rate of approximately 20 percent was observed, whereas the slide positivity rate in these countries 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. The same correlation was observed in Argentinean recruits; the serologic prevalence was approximately 5 percent, and the National 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 villages with high serologic indices than in the surrounding localities.

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. 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. 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. 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.

Serologic profiles of populations in Palawan, Philippines; in the sector Cuiaba, State of Mato Grosso, Brazil; and in Balihar, Bangladesh were determined. 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.

If eradication in the East Pakistan locality had failed, antibodies should have been detected in the young children. We demonstrated this by measuring antibody profiles in two communities in Afghanistan. Both in Saidabad and Bulla Quchi, malaria was reported to have been eradicated. A survey made 3 years ago showed the absence of antibodies in the 1- to 4-year-old group in Saidabad but the presence of antibodies in this age group in Bulla Quchi. After this study, recurrence of malaria transmission in the Bulla Quchi area in northeastern Afghanistan was confirmed, and antimalaria measures had to be reinstituted.

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.

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.

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 measure 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.

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

The few pilot field studies that have been carried out to test the operational use of serologic methods in malaria programs have been very promising. However, progress in this important aspect in the development of the techniques has been difficult because of the need to be dependent on 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.

To realize the full potential of the seroepidemiologic methodology, it is important for 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 most effective 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 field studies will also enable a group, such as ours, who are developing seroepidemiologic methods to indicate to the participating Malaria Eradication Programs how to use the serologic method to meet their specific needs.

Our studies have demonstrated 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.

The more advanced the stage of a malaria program, the greater is the need for alertness and vigilance. But, as malaria becomes a less prominent problem, the financial resources needed to carry out surveillance are often increasingly limited. At this stage, the use of serologic methods will be especially advantageous. Certain epidemiologic tasks, such as surveillance in areas of high vulnerability and receptivity, identification and coverage of populations at special risk to malaria, and screening of potential carriers to prevent introduction of malaria, can then be carried out more effectively.

The seroepidemiologic method can be usefully applied in areas where the endemicity of malaria is very low or where years of attack measures have not completely interrupted transmission. In such communities, it can profitably be used as an additional surveillance tool. Fewer blood specimens will need to be examined, and the many technicians who each year examined large numbers of slides can be diverted to areas where malaria is still a significant problem.

Another application of the serologic technique is for evaluation of the status of malaria. Evaluation is usually based on analyzing recorded surveillance data rather than on direct measurement of the level of malaria transmission, whereas, serology can offer an independent procedure for such an evaluation by examining a relatively small sample of the population.

The value of the seroepidemiologic method is that it can provide a sensitive additional tool to estimate the population's experience with malaria. Properly applied, it will permit interpretation of the level of transmission and identification of the subpopulations that are principally affected and will make possible estimating the effects of antimalaria measures. Use of this method requires only a small laboratory staff. One trained serologist and a small staff of technicians can operate a serologic laboratory that can process several thousand blood specimens a day.

In addition to an ongoing laboratory program for the technical improvement of all types of testing procedures, there is a need to evaluate the use of seroepidemiologic techniques in countries with different types of malaria in order to better define its optimal application in different situations. Toward this end, we have entered into longitudinal studies in South America and Nigeria. Malaria is not the same disease the world over, and one cannot assume that the criteria used in estimating the transmission of malaria in a country with a low slide positivity rate and predominantly *P. vivax* malaria, will be identical to a country like Nigeria with a very high level of transmission and a preponderance of *P. falciparum* infections.

The serologic method has to be given full evaluation and pilot implementation. It provides a new and sensitive tool for the surveillance of malaria and represents an additional factor for the