

AGENCY FOR INTERNATIONAL DEVELOPMENT
 WASHINGTON, D. C. 20523
BIBLIOGRAPHIC INPUT SHEET

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1. SUBJECT CLASSIFICATION	A. PRIMARY Public Health
	B. SECONDARY Malaria

2. TITLE AND SUBTITLE
 Serologic diagnosis of malaria; progress report, Jan.-Apr. 1969

3. AUTHOR(S)
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 (101) Public Health Service

4. DOCUMENT DATE 1968	5. NUMBER OF PAGES 8 p.	6. ARC NUMBER ARC
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7. REFERENCE ORGANIZATION NAME AND ADDRESS
 Public Health Service, Center for Disease Control, Parasitology Branch,
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8. SUPPLEMENTARY NOTES (*Sponsoring Organization, Publishers, Availability*)
 (Research Summary)

9. ABSTRACT
 The purpose of this project is to develop simple, rapid, accurate methods for the serologic diagnosis of malaria. Efforts have been concentrated on evaluating and standardizing the indirect hemagglutination technique (IHA) for the detection of malaria antibody for epidemiological studies, and the indirect fluorescent antibody test (IFA) for diagnostic and serologic speciation of malaria. With respect to the IHA test, the initial goals of the program have been achieved, in that the test has been standardized and shown to be a reproducible, sensitive, and specific technique for the diagnosis of malaria. Filter-paper collection of blood has been designed and evaluated, and has been used for the past year with excellent results. Blood on filter paper, collected in Nepal, Panama, Ethiopia, and other parts of the world, has arrived at the U.S.P.H.S. Center for Disease Control in Atlanta in excellent condition for titration. Application of the IHA test for seroepidemiologic purposes has been initiated. Studies have been designed to answer such question as whether the IHA procedure for measuring malarial antibody in a population correlate with parasitologic methods of malaria surveillance, what the duration of the hemagglutination antibody response is, whether the IHA test can be used to delineate the transmission zone of malaria, and whether it can be used to detect focal outbreaks of malaria in endemic areas. With respect to the IFA studies, a thick smear antigen has been employed and extensively evaluated. The IFA test now available is 95% sensitive and about 99% specific on a day-to-day basis, using four-fold dilutions. Using homologous antigen for human malaria, the IFA test will correctly speciate malaria antibody 84% of the time, and will give false positives in less than 5% of the samples tested.

10. CONTROL NUMBER PN-AAC-640	11. PRICE OF DOCUMENT
12. DESCRIPTORS	13. PROJECT NUMBER
	14. CONTRACT NUMBER PASA RA(HA)-5-68 Res.
	15. TYPE OF DOCUMENT

PASA. RA(HA)-5-68 Res.
PN-AAC-690

Serologic Diagnosis of Malaria

Progress Report

April 17, 1969

Submitted by

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PASA Control No. RA(HA) 5-68
PIO/T 931-11-990-485-72-3161104
Serologic Diagnosis of Malaria

I. Background

II. Purpose and Scope of Project:

The project is designed to develop simple, rapid, accurate methods for the serologic diagnosis of malaria which would be applicable in the worldwide malaria eradication program.

Toward this end, this laboratory has concentrated its efforts on the evaluation and standardization of the indirect hemagglutination technique (IHA) for the detection of malaria antibody for epidemiological studies and the indirect fluorescent antibody test (IFA) for diagnostic and serologic speciation of malaria.

III. Studies on the IHA test - Progress to Date:

The initial goals of the program have been achieved in that the indirect hemagglutination test has been standardized and shown to be a reproducible, sensitive and specific technique for the diagnosis of malaria (Rogers, W. A., Fried, J. A., and Kagan, I. G. A modified indirect microhemagglutination test for malaria. Am. J. Trop. Med. 17: 804-809, 1968).

Filter paper collection of blood has been designed and evaluated and has been used for the past year with excellent results. Blood on filter paper, collected in Nepal, Panama, Ethiopia, and other parts of the world, has arrived in Atlanta in excellent condition for titration.

With the purchase of a new Ribicell fractionator the production of crude antigen has been greatly facilitated, but problems associated with stability, lability, and purification have not been overcome.

During the current fiscal year the initial phases of the application of the IHA test for seroepidemiologic purposes

have been initiated. Toward this end, specific questions have been posed, and studies have been designed to evaluate the points raised.

- a. Does the IHA procedure for measuring malarial antibody in a population correlate with parasitologic methods of malaria surveillance?

Four collections of sera of young military recruits in the United States, Brazil, Colombia, and Argentina were studied. Each collection consisted of approximately 3000 sera from all parts of the country and represented a cross-section of the young male population. Ninety-nine percent of the U. S. collection was negative. This finding confirms the absence of malaria in this country.

In Brazil and Colombia, a serologic prevalence rate of approximately 20% was observed, but the slide positive rate was only 3-5%. However, when the states having the highest serologic prevalence rate were ranked with states having the highest slide positive rates, 80% correlation was observed. The same correlation was observed in Argentina where the serologic prevalence was approximately 5%, and the slide positive rate was less than 1%.

In a study of approximately 3000 sera collected for a yaws survey in the Philippine Republic, the absence of malaria in the islands of Leyte and Panay, observed in our study, correlated well with the malaria surveillance data.

- b. What is the duration of the hemagglutination antibody response?

In a study made in Pina, Panama, where malaria eradication procedures have been used for many years, positive serologic reactors had negative blood smears (taken at 2 month intervals) for as long as 5 years.

In 1954, 84 sera were collected from Tobago; of these, 80% were positive, with a geometric mean titer of 1:75. In 1969, sera were obtained from 23 of the individuals titered in the 1955 survey; of these sera, 17% were positive, with a mean titer of 3.3.

Antibody persists for at least 5 years and disappears slowly.

- c. Can the IHA test be used to delineate the transmission zone of malaria?

In Nepal the question of whether malaria was transmitted above 4000 feet was investigated. In a cluster of villages above 4000 feet, 163 sera were collected. Of the 22 individuals who were found to be positive, nineteen were males with a history of travel to the valley and into a malarious zone; 3 were young women who did not admit to visits to the valley. We were told that these women would be reluctant to admit that they left the village (and they probably did).

In Ethiopia, only 22% of the sera collected from residents above 6000 feet were positive; whereas 58% of the sera from individuals residing below 6000 feet were positive.

- d. Can the IHA test be used to detect focal outbreaks of malaria in endemic areas?

In the Philippine study, on the island of Cotabato, the prevalence of malarial IHA antibody ranged from 10 to 56%. Single villages with high positive rates (30-56%) could be found in geographical clusters of villages with rates ranging from 10-15%. These data suggest that in the villages with high serologic prevalence, active transmission is taking place. These malaria "hot spots" can be readily detected.

In Ethiopia serum samples taken in two locations in both wet and dry seasons showed that positivity rate and mean titer increased markedly in the wet season, the time of peak transmission. This indicates the potential of the IHA test for monitoring seasonal changes in malaria transmission.

- e. Is there a relationship between the mean geometric titer in a population and endemicity of malaria?

To date, approximately 20,000 sera have been titrated representing collections from the Western Hemisphere, Africa, and Asia. These are all "grab samples" and represent many types of populations and epidemiologic situations. The number is large, however, and many of the biases may cancel one another. When we plot the geometric mean titer on a logarithmic scale versus the percent positive in the collection, a straight line relationship is noted. Serial specimens drawn in a single area, but under differing epidemiological situations, move along this line in a predictable manner. This suggests to us that if we collect enough samples from an area we can readily

characterize it roughly into hypoendemic, endemic, or holoendemic. Serial specimens will allow an assessment of changing malaria incidence in an area.

Future work plans:

1. We plan to continue study of areas where malaria has been eradicated to learn more exactly when antibody titer disappears. The island of Cyprus has been recommended as a potential area for this type of study.
2. In collaboration with the NCDC field station in El Salvador, we are testing 8000 sera in a study on chemoprophylaxis. Since intensive malariometric methods will be used to study the various areas under test, we will be able to further evaluate the usefulness of the serologic method in characterization of malaria in an endemic zone.
3. In collaboration with Dr. Saave in New Guinea, we are studying the prevalence of antibody in a holoendemic area, before, during, and after eradication methods have been initiated. To date, approximately 1000 sera have been titrated.

On the technical side, studies on the use of formalinized cells and the preparation of a lyophilized antigen will be made. The Aztec autotitration machine will be evaluated for the rapid titration of samples.

Fluorescent Antibody Studies (IFA):

Since the initiation of our IFA studies, a thick smear antigen has been employed. This type of antigen has been extensively evaluated (Sulzer, A. J., Wilson, M., Hall, E. C. A thick-smear antigen in the IFA test for malaria. Am. J. Trop. Med. 1969).

The IFA test in our hands is 95% sensitive and about 99% specific on a day to day basis using 4-fold dilutions.

a. Speciation studies:

Using homologous antigen for human malaria, the IFA test will correctly speciate malaria antibody 84% of the time. The test will give false positives in less than 5% of the samples tested.

b. Adaptation of human malaria to simian hosts:

Since homologous human malaria antigen has to be used, it is important to have readily available sources of antigen. Toward this end, P. vivax and P. falciparum have been established in the laboratory in Aotus trivergatus (Owl) monkeys. P. malaria has not been established in Aotus, but this species may be available to us in the near future. In its absence we have evaluated P. brasilianum maintained in Ateles sp. (Spider) monkeys. Evaluation of this simian malaria suggests that it can be used in place of P. malariae. To establish P. ovale in the laboratory, 2 chimpanzees are currently under study. In the first animal infected with P. ovale a titer has been observed and only a transient appearance of parasites was noted. The second animal has just been splenectomized and will be infected.

c. Studies on the duration of antibody titer:

This study was made with 69 Vietnam returnees who relapsed with malaria after returning to this country and who upon entry into the military hospital, received curative chemotherapy. Titration of several sera from each individual established that titers fall to 1:16, or are negative within 6 months in 90% of the cases studied. Study of a large group of other returning veterans indicates that past history of malaria does not influence the IFA titer.

Future work plans:

Two reactions have been noted in positive sera. The titer with trophozoites is lower than the titer observed with schizonts. The significance of this observation will be investigated.

Further studies will be made on the P. brasilianum - P. malaria and P. Schwetzi - P. ovale complex in an effort to ascertain whether the simian malaras can be effectively used for antigen in the IFA test.

Work with the Space Division of the Aerojet-General Corporation in a program for automation of the malaria IFA and the SAFA (soluble fluorescent antibody antigen) tests will be initiated.

IV. Approval of Research Resources and Budget:

The application of the IHA test for epidemiologic surveillance and characterization of malarial areas will require several years of field evaluation in order to define and characterize the parameters and limits of the test. Under the current budget, the program outlined and projected for this phase of the work is adequate. When the program was formulated, a request for an additional professional person in FY-1970 was requested. This position called for a Biochemist who would work on the purification and the preparation of specific malarial antigens. The IHA test is a workable procedure, and there is no reason to doubt that it will be useful in the malaria eradication effort. The production of specific stable reagents that can be employed in the field laboratory is essential for the ultimate success of this project. Dr. Mathews, who is in charge of the IHA Malaria Laboratory, will devote much of his time to the epidemiologic aspects of this project. A request for an increase in funds in FY-1970 for a biochemist-immunologist is important to the attainment of a reliable field serologic procedure utilizing specific stable and sensitive antigens.

The malaria program in the Parasitology Section receives support from NCDC funds. Dr. Alexander Sulzer, who is in charge of the fluorescent antibody laboratory, is not funded from AID sources. The animal caretaker for our monkey colony, laboratory maintenance, glassware washing, red cells, and auxiliary technical support is funded from NCDC sources.

As the program develops, a full time project director, would be beneficial to the success of the program. The professional biochemist or immunologist who will be recruited will be a person of demonstrated ability in malaria work and would be responsible for his own program and would also be coordinator of the fluorescent antibody and epidemiologic programs.

Revised Budget (FY-1970)Personnel compensation

GS-14 (1) Malariologist (6 mos.)	9,252
GS-12 (1) Dr. Mathews	13,389
GS-9 (1) Mrs. Fried (7-3 to 9-1)	9,320
GS-9 (1) Miss Wilson (7-2 to 9-1)	9,320
GS-4 (3) Miss Oliver (4-2 to 4-3)	5,590
<u>Total personnel compensation</u>	<u>46,871</u>

<u>Personnel benefits</u>	<u>3,749</u>
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Travel

One trip to El Salvador	600
One trip to Cyprus	1,000
One trip to New Guinea	1,500
<u>Total travel</u>	<u>3,100</u>

<u>Xerox rental (5¢ per copy) and telephone service</u>	<u>380</u>
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<u>Laundry (uniforms (1300) plus towels</u>	<u>700</u>
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Supplies

Rhesus monkeys (100 @ \$50)	5,000
Chimpanzees (2 @ \$700)	1,400
Aotus (40 @ \$40)	1,600
Ateles (5 @ \$40)	200

Miscellaneous lab supplies purchased from outside sources	500
Glassware	5,000
Blood Products	700
Media	800

<u>Total supplies</u>	<u>15,200</u>
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<u>Total Direct Cost</u>	<u>70,000</u>
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Plus 20% indirect cost*	14,000
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<u>Total Cost (excl. Aerojet subcontract)</u>	<u>\$84,000</u>
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*Subject to change at beginning of each fiscal year.