

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

FOR AID USE ONLY

*Batch 39*

1. SUBJECT CLASSIFICATION	A. PRIMARY	TEMPORARY
	B. SECONDARY	

2. TITLE AND SUBTITLE  
Serologic diagnosis of malaria; progress report, March-June, 1970

3. AUTHOR(S)  
(100) Kagan, I.G.  
(101) HEW/PHS

4. DOCUMENT DATE 1970	5. NUMBER OF PAGES 8p.	6. ARC NUMBER ARC
--------------------------	---------------------------	----------------------

7. REFERENCE ORGANIZATION NAME AND ADDRESS  
PHS

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

9. ABSTRACT

(HEALTH R & D)

10. CONTROL NUMBER PN-AAC-643	11. PRICE OF DOCUMENT
12. DESCRIPTORS	13. PROJECT NUMBER
	14. CONTRACT NUMBER PASA RA(HA)-5-68 Res.
	15. TYPE OF DOCUMENT

PASA PA-7111-5-00 RES.  
PN-AAC-643

QUARTERLY REPORT

March 31-June 30, 1970

Submitted by  
Dr. Irving G. Kagan  
Chief, Parasitology Section  
Center for Disease Control  
Atlanta, Georgia 30333

## SEROLOGIC DIAGNOSIS OF MALARIA

PASA Control No. RA (HA) 5-68

PIO/T 931-17-511-485-72-3106759

Amendment #4

### Purpose and Scope of Project

Program is designed to develop simple, rapid, sensitive, and specific serologic methods for the diagnosis of malaria which are applicable for epidemiologic and clinical purposes.

In an effort to develop methods that may be applicable in the world-wide malaria eradication program for surveillance and other purposes, this laboratory has concentrated its effort on the evaluation and standardization of the indirect hemagglutination (IHA) technique for the detection of malaria antibody. Studies on the indirect fluorescent antibody (IFA) test are being made to define the extent of cross reaction and sensitivity of speciation.

#### A. Studies on the Indirect Hemagglutination (IHA) Test

##### I. Antigen Studies

Procedures modified from those of Chavin (Milit. Med., 131: 1124, 1966) and Wellde et al. (Milit. Med., 134: 1284, 1969) have been employed in attempts to isolate antigens from lysates of red blood cells infected with Plasmodium knowlesi. This technique, involving precipitation with rivinol, has produced an antigen with greatly increased stability. This antigen has been lyophilized with success and remains stable for at least 6 weeks when the lyophilized material is held at room temperature. Studies on the sensitivity and specificity of this antigen preparation are presently under way.

Investigation has continued on the use of gluteraldehyde as a stabilizing agent for human group "O" erythrocytes. Cells treated in this manner can be satisfactorily sensitized following storage at 4° C for at least 4 months. Studies are now in progress to determine if these cells can be sensitized with antigen and lyophilized while maintaining all their activity.

## II. Seroepidemiologic Studies

### a. Haiti:

Dr. Mathews traveled to Haiti in April and collected a total of 677 sera and filter paper bloods from persons living in areas of high, intermediate, and low malaria endemicities. This serologic evaluation confirmed the patterns of malaria distribution and indicated one area where occult malaria may be occurring. Plans for confirmation of this observation are being implemented. Further studies on the epidemiology of malaria in Haiti are being planned.

### b. Pakistan:

Studies in collaboration with Dr. M. Rahman in East Pakistan have been initiated. Over 1,300 filter paper samples have been tested to date. These samples originated in two areas: one with a 4-year control program and one where the control program is being initiated. These studies will continue and will prove invaluable in demonstrating the serologic response in populations where malaria is being controlled.

### c. New Guinea:

Studies with Dr. J. J. Saave in New Guinea are continuing. The quantity and quality of data provided by Dr. Saave have necessitated the use of the computer facilities for processing the data. Programs for this are being prepared and some of the analyses should be available in the near future.

### d. Brazil:

Approximately 1,500 samples from the interior of Brazil have been tested in conjunction with the Central America Malaria Research Station (CAMRS) and the Malaria Program, CDC. These studies demonstrated a very low level of malaria in an area under consideration for studies by CAMRS. The serologic results were confirmed by slide examination, and this program is being re-evaluated.

### e. Philippines:

Protocols for a large-scale serologic survey of malaria in the Philippines are currently being prepared. This study will be conducted to assess the distribution of malaria as determined by serologic and classical methods.

## B. Fluorescent Antibody Laboratory

### I. Evaluation and Development of the Indirect Fluorescent Antibody (IFA) Test for Malaria

- a. Efforts are being made to obtain 2 chimpanzees from the Air Force to maintain Plasmodium ovale for antigen. At the present time we do not have a source of this species. Facilities to house the animals (cages and animal room) are being prepared.
- b. Studies on the significance of high malarial antibody titers in students residing in the United States, with a history of lifetime residence in endemic areas for malaria, have been extended. Individuals with high titers not treated for malaria have maintained their titers for 2 years. Thirty foreign students with malaria titers have received curative treatment and will be tested at various time intervals for 2 years. If these individuals do have cryptic malaria as indicated by the consistent titers of the untreated group, their titers should be reduced after curative treatment.
- c. A guest researcher, Dr. Colin Ludford sponsored by the Australian Meat Institute, is now in the laboratory investigating cross reactions between Babesia and Plasmodium. Initial investigations indicate a great deal of cross reactivity between the two genera. The significance of this serologic activity will be evaluated with regard to the diagnosis of malaria by FA.
- d. Significance of malaria antibody titer in the U. S. nationals with long residence in areas endemic for malaria is being assessed. A battery of 100 sera from U. S. missionaries living in endemic areas were received from Dr. J. Frame, a medical missionary physician in New York. Only 5 of the 100 individuals had positive serologic reactions. These persons will be treated and followed serologically to determine if antibody titers diminish after treatment.

### II. New Studies Being Planned

A cooperative project with the Southwestern Foundation for Research and Education, San Antonio, Texas, is planned for further work on Hepatocystis, a blood parasite of the baboon closely related to malaria, and its cross reactions with Plasmodium.

### C. New Developments in the Program

To assist the epidemiologic aspects of this program, a biochemist, Dr. David Farshy, has been detailed for 3 months to the Malaria Serology Laboratory. Dr. Farshy will be given the responsibility of preparing stable gluteraldehyde-sensitized red cells for serologic studies.

Dr. Hans Lobel has been detailed to the Malaria Serology Laboratory in the role as a medical epidemiologist. Dr. Lobel will assist in designing field studies, in preparing computer programs for processing and handling serologic data, and assist in evaluation of the filter paper method.

More emphasis is being placed on designing field studies to evaluate the various parameters of the hemagglutination test.

### D. Publications:

#### Published:

Kagan, I. G., H. M. Mathews, W. A. Rogers, and J. Fried. 1970. Seroepidemiologic studies by indirect hemagglutination tests for malaria. I. Military recruit collections from the United States, Brazil, Colombia, and Argentina. Bull. W.H.O.

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

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

#### Accepted for Publication:

Mathews, H. M., G. U. Fisher, and I. G. Kagan. The persistence of malaria antibody in Tobago, West Indies, following eradication as measured by the indirect hemagglutination test. Am. J. Trop. Med.

Wilson, M., A. J. Sulzer, W. A. Rogers, J. A. Fried, and H. M. Mathews. Comparison of the indirect fluorescent antibody and indirect hemagglutination tests for malaria antibody. *Am. J. Trop. Med. Hyg.*

Gleason, N. N., M. Wilson, A. J. Sulzer, and K. Runcik. Agreement between slide diagnosis and IFA serology in Plasmodium vivax and P. falciparum infections. *Am. J. Trop. Med. Hyg.*

Submitted for Publication:

Mathews, H. M., J. A. Fried, and I. G. Kagan. A sero-epidemiologic study of malaria in the Republic of the Philippines by the indirect hemagglutination test. *Am. J. Epid.*

## Current Work Plan

The indirect hemagglutination (IHA) test is being developed primarily to measure the intensity and distribution of malaria in countries where the disease is a public health problem. This project will have three major goals in FY 1971.

### A. The improvement of Antigen Stability.

The antigen preparation presently in use in the laboratory is very labile and is not suitable for use in the field. Stabilization of the antigen will allow wider application of the IHA test as a field tool. Preliminary studies have indicated that selective precipitation of antigenic components with the compound rivinol results in a product which is reactive and reasonably stable. This work will be intensified, using gel filtration and ion exchange chromatography in conjunction with rivinol to produce a more stable antigen. These studies will require increased numbers of experimental monkeys for growth of Plasmodium knowlesi, additional materials, but no major equipment purchases are anticipated.

### B. The Development of Stable, Sensitized, Red Blood Cells Which Can Be Used Under Field Conditions.

A number of aldehydes have been found to stabilize human type "O" red blood cells. Stable red blood cells which can be coated with antigen and preserved by drying or freezing would enhance the usefulness of the IHA test under field conditions. A biochemist, Dr. David Farshy, has been detailed to the Malaria Serology Laboratory for 3 months to work on the stabilization problem. The plan of work will involve the use of gluteraldehyde-treated red blood cells in conjunction with experimental antigen fractions under development.

### C. Continued Field Evaluation of the Serologic Method of Malaria Surveillance.

Field evaluations are being carried out in New Guinea, Brazil, Haiti, East Pakistan, and the Philippines to determine the efficacy of the IHA test in relation to the traditional malarimetric methods by which present anti-malaria efforts are guided. These studies are being developed to demonstrate serologic profiles of persons living in areas with varying endemicities of malaria and in various phases of eradication. The 3 specific problems that will be dealt

with in these studies are: 1) the persistence of malaria antibodies in persons living in areas which have been freed of malaria; 2) the delimitation of areas where malaria transmission occurs, and 3) the significance of mean titer levels in populations with varying malaria endemicities. Further studies will be carried out using the filter paper collection method to further quantitate its usefulness in field collections.

Implementation of field studies will require increased travel by the staff of the Malaria Serology Laboratory. Dr. Hans O. Lobel, a medical epidemiologist, has been detailed to the laboratory to serve as liaison between field and laboratory personnel. Field studies will require on-site inspection of conditions, review of records, consultation with field personnel, and collection of special material for evaluation.

Increased data production from field studies has necessitated the development of a data processing system utilizing available computer facilities. This system will become operational within the next year and will result in faster, more accurate interpretation of field data.