PD-AAQ-049 36871

AGENCY FOR INTERNATIONAL DEVELOPMENT

PROJECT DATA SHEET

1. TRANSACTION CODE: A = Add, C = Change, D = Delete — Amendment Number

DOCUMENT CODE: 3

2. COUNTRY/ENTITY: S&T Interregional

3. PROJECT NUMBER: 931-0453.45

4. BUREAU/OFFICE: S&T/H/CD — 10

5. PROJECT TITLE (maximum 40 characters): Malaria Immunity - KT & R Laboratories

6. PROJECT ASSISTANCE COMPLETION DATE (PACD): MM 12 DD 31 YY 87

7. ESTIMATED DATE OF OBLIGATION (Under 'B.' below, enter 1, 2, 3, or 4)
A. Initial FY 85 B. Quarter 2 C. Final FY 87

8. COSTS ($000 OR EQUIVALENT $1 =)

A. FUNDING SOURCE	FIRST FY B. FX	C. L/C	D. Total	LIFE OF PROJECT E. FX	F. L/C	G. Total
AID Appropriated Total	235		235	754		754
(Grant)	(235)	( )	(235)	(754)	( )	(754)
(Loan)	( )	( )	( )	( )	( )	( )
Other U.S. 1.						
Other U.S. 2.						
Host Country						
Other Donor(s)						
TOTALS	235		235	754		754

9. SCHEDULE OF AID FUNDING ($000)

A. APPROPRIATION	B. PRIMARY PURPOSE CODE	C. PRIMARY TECH. CODE 1. Grant	2. Loan	D. OBLIGATIONS TO DATE 1. Grant	2. Loan	E. AMOUNT APPROVED THIS ACTION 1. Grant	2. Loan	F. LIFE OF PROJECT 1. Grant	2. Loan
(1) ST/H	540	542				754		754	
(2)									
(3)									
(4)									
TOTALS						754		754	

10. SECONDARY TECHNICAL CODES (maximum 6 codes of 3 positions each)

11. SECONDARY PURPOSE CODE

12. SPECIAL CONCERNS CODES (maximum 7 codes of 4 positions each)
A. Code
B. Amount

13. PROJECT PURPOSE (maximum 480 characters)

This project will conduct research to develop a simple immunodiagnostic test for detection of malaria at the clinical and subclinical (latent) levels of the disease.

14. SCHEDULED EVALUATIONS
Interim MM 12 YY 85 MM 12 YY 86 Final MM 12 YY 87

15. SOURCE/ORIGIN OF GOODS AND SERVICES
☐ 000 ☐ 941 ☐ Local ☐ Other (Specify)

16. AMENDMENTS/NATURE OF CHANGE PROPOSED (This is page 1 of a ____ page PP Amendment.)

17. APPROVED BY
Signature: Ann Van Dusen
Title: Dr. Ann Van Dusen, Acting Director, S&T/H
Date Signed: MM 11 DD 06 YY 84

18. DATE DOCUMENT RECEIVED IN AID/W, OR FOR AID/W DOCUMENTS, DATE OF DISTRIBUTION
MM DD YY

AID 1330-4 (8-79)

PROJECT AUTHORIZATION

Name of Entity: Interregional

Project Title: The Development of a Simple Immunodiagnostic Test for Malaria.

Project No.: 931-0453.45

Grantee: KT and R Laboratories, Inc. Stillwater, Minnesota

1. Pursuant to Section 104 of the Foreign Assistance Act of 1961, as amended, I hereby authorize the research project entitled "The Development of a Simple Immunodiagnostic Test for Malaria" involving not to exceed $754,000 of S&T Bureau grant funds over a three year period from the date of authorization subject to the availability of funds in accordance with the A.I.D. OYB/Allotment process to help in financing the costs of the project.

2. The project will conduct research to develop a simplified immunodiagnostic for detection of human malaria at the clinical and subclinical (latent) levels of the disease to aid in the future conduct of malaria vaccine clinical trials in the developing world.

Clearances:
A. S&T/H, A. Van Dusen _____ date 11-6-84
B. S&T/PO. G. Eaton _____ date _____

S&T/HP, James Sarn, M.D.
Agency Director
for Health and Population

12-3-84
(Date)

ACTION MEMORANDUM FOR AGENCY DIRECTOR
FOR HEALTH AND POPULATION

FROM: S&T/H, A. Van Dusen

SUBJECT: Selection of Assistance Recipients for the Malaria Immunity and Vaccination Research Program

Problem: Your approval is required to negotiate a cooperative agreement on the basis of an unsolicited proposal without inviting applications from other entities.

Discussion: The Office of Health, S&T, is in receipt of an unsolicited proposal for the development of an enzyme linked immunosorbent assay (ELISA) which will detect actual plasmodial infection during both clinical and subclinical (latent) forms of the disease.

The project has been reviewed by A.I.D.'s external expert panel in accordance with FPR 1-4.909, and FPR 1-4.910(b) and (c). The proposal contains a number of proprietary products and methods, especially the specific methodology for the antigen binding immunoassay. These monoclonal antibodies and research methodologies are fundamental to the research program and cannot be obtained by A.I.D. from any other source. This prohibits the conduct of a market search for this procurement per FPR 1-4.911. The unsolicited research proposal is from:

KT and R Laboratories, Stillwater, Minnesota

The importance of this project to our malaria vaccine program cannot be overemphasized, especially as we accelerate the program under several directives from the Administrator.

Recommendation: Pursuant to Handbook 13, Chapter 1B2(e) and the above cited FPR's, we recommend that this institution be selected as the assistance recipient to carry out the program outlined in their respective unsolicited proposal. We ask that you approve the negotiation of cooperative agreement without inviting applications from other entities. Our recommendation is based both on our review of the unsolicited proposal and our determination that the proposers are the most appropriate institution to carry out the proposed program in malaria research and training.

Approved _____

Disapproved _____

Date 12-2-84

Clearances:
S&T/H, W. Oglesby _____ Date 2 Nov 84
S&T/PO, G. Eaton _____ Date _____

October 31, 1984

MEMORANDUM

TO: CM/COD/PE, Ms. Judith Johnson

FROM: S&T/HP, James Sarn, M.D.

SUBJECT: Justification for non-competitive procurement of unsolicited proposal from the KT and R Laboratories, Inc. entitled "The Development of a Simple Immunodiagnostic Test for Malaria"

The subject research proposal is an unsolicited proposal. The substance of the proposal is not available to the Government without restriction from another source, nor does it resemble any pending competitive solicitation. The substance is sufficiently unique to justify acceptance as an unsolicited proposal.

The project officer certification with reference to A.I.D. PR Notice 78-4 follows:

> I certify that neither I nor, to the best of my knowledge and belief, any other A.I.D. employee solicited the proposal or had any prior contact with the proposing institution, other than to convey interest in the field of malaria immunity and vaccination relative to the efforts described in the unsolicited proposal.

James M. Erickson

Dr. James M. Erickson, Project Manager

I request that you award this contract on a non-competitive basis to the KT and R Laboratories, Inc. without consideration of other sources.

Clearances:
S&T/PO, G. Eaton _____ date _____
S&T/H, A. Van Dusen _____ date _____

ACTION MEMORANDUM FOR THE AGENCY DIRECTOR
FOR HEALTH AND POPULATION

FROM: S&T/H, Ann Van Dusen

Action: Your approval is requested for a grant of $754,000 from Section 104 of the Foreign Assistance Act of 1961 as amended for project 9312-0453.45, Malaria Immunity and Vaccination Research, the KT and R Laboratories, Stillwater, Minnesota.

Discussion: Attempts to find a vaccine against malaria began in the 1930's but were supplanted by new drugs developed during World War II and later followed by world-wide anti-malaria programs based upon adulticiding with DDT. Resistance to drugs and insecticides, lack of money and political instability, have reduced the prospects of malaria eradication and have stimulated renewed interest in a malaria vaccine.

In the past several months, several major advances have been achieved in the sporozoite and merozoite blood stages of *Plasmodium falciparum* and *P. vivax*. This research has brought A.I.D. to a point where large scale primate trials are required of prototype vaccines. Human trials may begin soon after advanced non-human primate trials if the data generated utilizing *Aotus* monkeys are fully acceptable to FDA. Of critical importance for field clinical trials is a simple microimmunodiagnostic test to measure specific blood plasma circulating antigens of the various human malaria species. The test must be rapid, sensitive, specific, simple and low in cost. Current biomedical technologies suggest that a micro-enzyme linked immunosorbent assay (ELISA) as a DOT system on nitrocellulose will allow the development of the required field assay. This proposal from KT & R Laboratories will focus on part of the immunodiagnostic development so very critical to the A.I.D. malaria program acceleration.

Justification to the Congress: Project Funding is included in the FY 1985 Congressional Presentation, Annex V, Centrally funded Programs, page 53.

Clearances Obtained: This project was approved by the A.I.D. external expert panel on the basis of scientific methodology, qualifications and experience of the proposed investigators, and the institutional capability in accordance to FPR 1-4.909, FPR 1-4.910 (b) and (c), and FPR 1-4.911.

Recommendations:
1. That you sign the attached authorization.
2. That you sign the attached justifications for non-competitive procurement.

Clearance:
S&T/PO, G. Eaton _____ Date _____

Attachments:
1. Project Authorization
2. Non-competivie Justifications for Cooperative Agreement
3. Project Paper

CAUTION
Contains Proprietary Information

RESEARCH PROPOSAL

"DEVELOPMENT OF A SIMPLE IMMUNODIAGNOSTIC
TEST FOR MALARIA SUITABLE FOR
PERFORMANCE IN THE PHYSICIAN'S OFFICE"

NOTE

**This document contains
patentable and/or copyrightable
information not for public disclosure**

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I. SUMMARY OF THE PROPOSED STUDY

A novel and simple microimmunodiagnostic test for malaria which can be performed by a physician, even under field conditions, is proposed. The test is based upon micro-enzyme-linked Immunosorbent Assay (ELISA) for detection of specific blood plasma circulating antigen. The attributes of the test include: rapidity, sensitivity, specificity, simplicity and cost-effectiveness. In the first phase of the study, the test will be developed and optimized in the Bolivian Squirrel Monkey (*Simiri scuireus*) to which *Plasmodium falciparum* has been adapted. The acute course of experimental malaria blood phase infection in this animal model mimics the human clinical picture of *P. falciparum* malaria including the cerebral syndrome. The study will also include assays of free and complexed specific antigens in both mild and convalescent stages of malaria in order to standardize the optimal operational conditions according to the kinetic variation of antigen-antibody immune complex proportions. The results of these studies will then be critically evaluated using a series of serum samples from primary human *P. falciparum* infections now available to us.

The proposed version of ELISA is the DOT-ELISA system on nitrocellulose membranes. For purposes of standardization the DOT-ELISA will be evaluated against the standard microplate ELISA. The following controls will be routinely included:

1. A plasma standard with a known strong positive reaction for P. falciparum antigen (free or complexed).
2. A standard giving an intermediate (moderate) reaction for antigen and immune complexes.
3. A standard giving a weak (borderline) reaction.
4. A negative plasma standard.

One microliter of test and standard samples will be dotted onto mitrocellulose membranes. Monospecific antiserum will then be added, followed by an enzyme conjugated antibody and finally by a precipitating substrate. The resultant color will be visually scored as strongly positive (4+); intermediate reaction (3+); weak positive (2+) or negative (0) by direct comparison with the reference antigen standards.

The proposed DOT-ELISA system will be the first diagnostic test suitable for detection of actual plasmodial infection during both clinical and subclinical (latent) forms of the disease. In this test, large numbers of samples may be processed within a 2-hour period. The test can be carried out at room temperature and can be read visually. The reagents used in DOT-ELISA are estimated to cost less than 5 cents per sample. The advantages of this procedure in comparison to the currently used parasitologic procedures for diagnosis of malaria are discussed.

The first phase of study (approximately 1½ years), using clinically defined human and monkey serum samples will be followed by application of the test to human populations residing in various regions of malaria endemicity. The results of the study will then be statistically analyzed for reproducibility, predictive value, sensitivity, specificity (with reference to actual infection with P. falciparum) and its practicability in the hands of physicians and medical technologists.

II. INTRODUCTION

During the last two decades the immunogenicity and antigenicity of various protozoal parasites, including malaria, have been extensively investigated (1, 2, 3, 4). However, attention has focused predominantly on antibody detection methods, which in strict terms, simply measure evidence of exposure to the etiologic agent. In most cases the presence of antibody cannot differentiate between current and previous infection. These methods, therefore, provide basically a presumptive diagnosis (4, 5, 6). The fundamental problem is that the measurable humoral immune response generally occurs long after initial exposure and persists beyond clinical cure.

Further, the absence of antibody response does not preclude infection in the presence of concomitant factors such as malnutrition, immunosuppression (5. 7), polyparasitism (7, 8),

antigenic variation and the complexing of antibody with its specific antigen.

Most of the diagnostic procedures for parasitic diseases have been geared to major diagnostic and research laboratories. The ordinary physician relies heavily, however, on the laboratory data to support clinical observations before a definitive diagnosis can be reached. For a timely, lifesaving diagnosis the physician should be able to perform the test in his office or on the bedside of the patient, thereby cutting down the cost of laboratory tests, minimizing the delay of the appropriate therapy and reducing the duration of hospitalization.

Currently, parasitologic methods are considered to be the most realiable techniques of diagnosis but they are time consuming and are liable to give false negative results in low intensity infections or situations of long prepatent periods.

Specific antigen detection methods, on the other hand, are based on metabolic products of the invading parasite (5,12, 13). Based on studies of various erythrocytic protozoan infections, including malaria, there is a direct correlation between the levels of parasitemia and circulating soluble plasma antigens (9, 10, 11, 12, 13). Free plasma soluble antigens would thus be diagnostically detectable before, during and after peripheral blood parasitemia is in evidence. When immune complexes form

in the zone of equivalence and antibody excess, detection of complexed antigen would be indicated. Thus, detection of free or complexed antigen would cut across the entire clinical profile of malaria including subclinical, early onset acute, convalescent and carrier states.

The following section will summarize the rational for the use of specific soluble antigens and specific antigen-antibody complexes as a scientifically sound diagnostic criteria.

III. THE SCIENTIFIC BASIS FOR THE PROPOSED STUDY

The role of soluble circulating antigens and immune complexes in the pathogenesis of plasmodial infections has been confirmed in a number of experimental models (14, 15), and in clinical human malaria (16, 17, 18). University of Illinois scientists were among the pioneers in demonstrating the protective and diagnostic value of these antigens in malaria (9, 10, 11, 12, 13). Adult human immune sera from the Gambia reacts with plasma from children with acute malaria (18), thus demonstrating the presence of soluble antigen in acute human malaria. The technique used in this study was the agar gel double diffusion, a rather insensitive method for routine use. Subsequently, Mackey, et al (19), developed a radioimmunoassay for P. falciparum antigens and later enzyme-linked immunosorbent assays were described for the detection of P. falciparum antigens in patient's erythrocytes or serum (20, 21). With

the development of improved ELISA techniques such as DOT-ELISA (22, 23), it should now be possible to detect antigen, antibody or immune complexes in nanogram quantities.

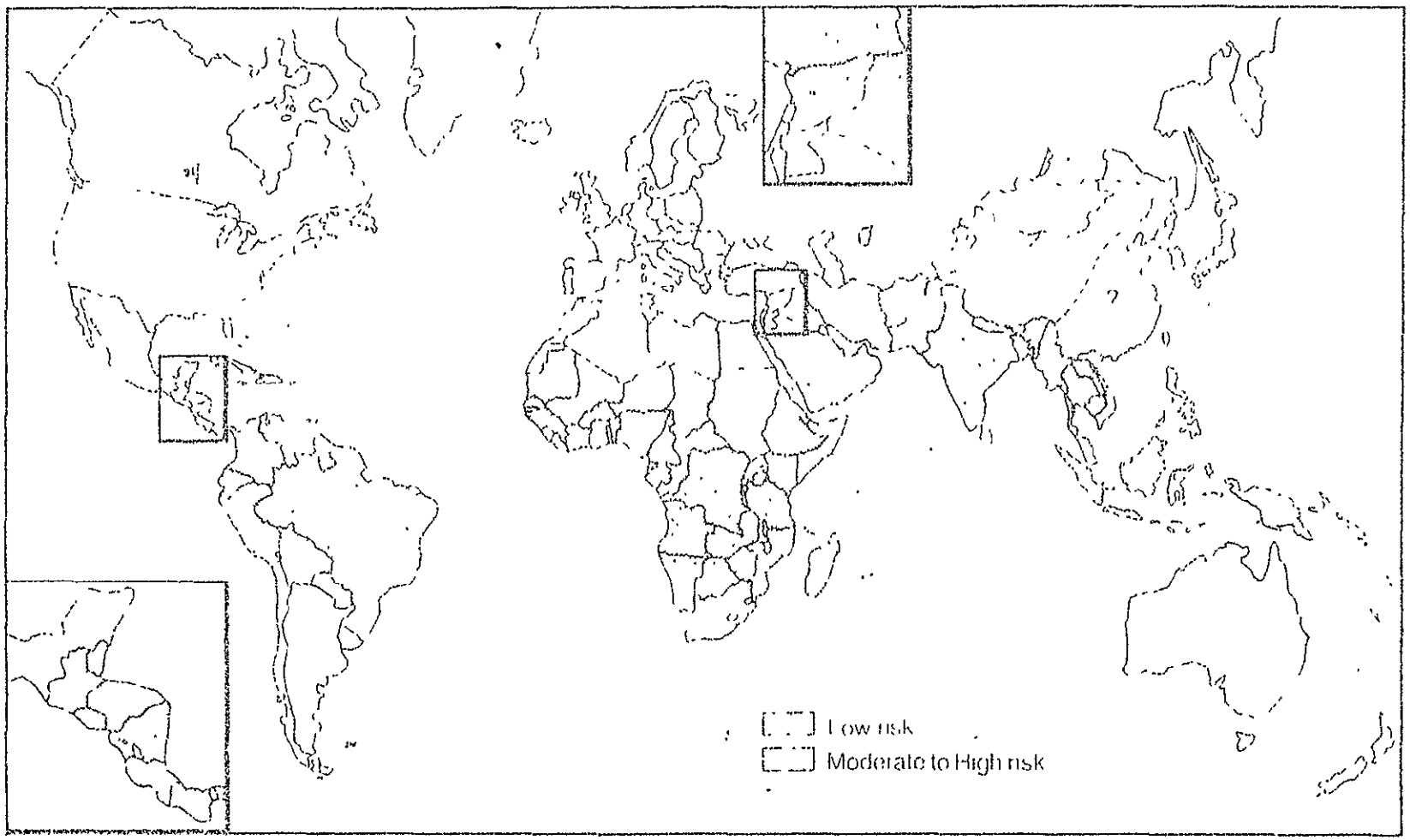
The DOT-ELISA system will be used throughout this study, first in the monkey model of malaria in correlation with samples from known primary human infections, and later in human populations resident in areas of varying degrees of malaria endemicity of Colombia and Venezuela.

IV. JUSTIFICATION

Malaria is a disease of great global importance, particularly in tropical and subtropical countries. Malaria causes approximately 200 million cases of morbidity and 3 to 5 million mortality casualities each year, and it is estimated that some 1,620 million inhabitants reside in regions where malaria is endemic to varying degrees (24, 25, 26, 27). Major efforts towards the development of malaria vaccines are underway in many laboratories throughout the world. However, neither vaccines nor chemotherapy can be rationally implemented by any medical team without adequate knowledge of the precise disease status of the respective patient and population. Consequently, the need for differential specific identification of current malaria infection is an essential prerequisite to the effective implementation of control measures. Within the United States itself,

imported malaria is of major public health and military importance (27, 28, 29) and early diagnosis is urgently required. According to reports from the Center for Disease Control (29), the number of imported cases in the United States escalated by over 100% among civilians in 1980. The same situation exists in other countries generally considered to be free from malaria (27). Also, deployment of Armed Forces from malaria-free regions to the endemic areas will demand a fast and accurate diagnostic method. Again, DOT-ELISA seems to be a very promising test for such early diagnosis. Figure 1 depicts the world-wide distribution of malaria. Like many South and Central American countries, Colombia and Venezuela have a high incidence and prevalence of human malaria (29). In general, all areas of Colombia are at risk except Bogota and its vicinity. Risk exists in rural areas of Urbaa, Bajo, Canca-Nechi, Magualena Medio, Caqueta, Sarare, Catatambo, Pacifico Central and Sub, Putumayo, Ariatic and Alto Vaupes. This malaria risk exists throughout the year among populations residing in altitudes below 800 meters. A major problem is that chloroquine resistant _P. falciparum_ has been shown in all malaria-endemic areas of Colombia. Thus, a simple sensitive, specific and cost-effective diagnostic method is urgently needed to facilitate rapid case identification and institution of appropriate therapy.

Fig 1 Areas of Risk for Malaria Transmission [1]



[1] Based on map published in W H O Weekly Epidemiologic Record, No 31, 1980.

Until only recently the neighboring country of Venezuela was considered free of malaria. During 1982 only 600 clinical cases were reported in that country. However, during 1983 the number of cases escalated to 25,000. Further information indicates that malaria is rapidly spreading in several other regions of Venezuela and currently constitutes a major concern of the newly elected government of that country (Personal communication: Dr. Andres Sucre, Director Malaria Control Program of Venezuela, 1984). This confirms the published CDC report (29) which states that many areas of Venezuela are at risk of chloroquine resistant malaria; in particular, rural areas of Terr., Fed., Amazons, Apure, Bolivia, Barinas, Tachira and Zulia. Further, according to Gabaldon (30), a single case introduced in a highly susceptible population may spread rapidly in surrounding villages. Due to a previously successful anti-malaria campaign a large population of Venezuela is extremely susceptible.

Just as in Colombia, a rapid and cost-effective diagnostic method is urgently needed in Venezuela in order to arrest the spread of malaria.

V. OBJECTIVES

The objective of this research proposal is to develop, evaluate and standardize the DOT-ELISA system as a definitive

diagnostic test for human P. falciparum malaria. The test is based upon detection of plasma soluble plasmodial antigens and specific immune complexes. The first phase of the study (approximately 1½ years) will utilize samples from primary human infections and those from squirrel monkeys infected with P. falciparum. The second phase of equal duration will consist of evaluation of the test under field conditions in Colombia and Venezuela, South America. Data gathered in the second phase of the study will be statistically analyzed for specificity, sensitivity, predictive value, reproducibility and practicability in the hands of physicians and medical technologists. It is anticipated that at the end of this study the DOT-ELISA technology will be ready for large scale field evaluation also in regions other than South America.

VI. MATERIALS AND METHODS

1. Plasmodium falciparum strains

Strains of P. falciparum from Southeast Asia, Africa and Central America, adapted to and propagated in Bolivian Squirrel monkeys, will be used throughout the study. These isolants represent a spectrum of chloroquine sensitive and chloroquine resistant P. falciparum organisms.

2. Experimental animals and sample collection

Male adult Bolivian Squirrel monkeys (Simiri scuireus), weighing between 600 and 1,000 grams will be used. Prior

to the experiment all monkeys will be screened by the indirect fluorescent antibody (IFA) test for evidence of previous exposure to Plasmodium spp. (P. falciparum and P. brasilianum). Monkeys positive in the IFA at 1:10 serum dilution shall be excluded from the experiment. Pre-inoculation plasma will be collected from all monkeys. Following infection, weekly samples for hematologic and immunologic studies will be collected. Urine samples will also be collected whenever possible. After centrifugation the plasma samples will be aliquoted and stored at -70° C until analyzed. Also, whole blood samples collected directly onto nitrocellulose membranes will be preserved for later use in the DOT-ELISA.

3. Clinical categories of experimental monkeys

Experimental monkeys will be divided into 4 categories:

a. Clean monkeys: uninfected monkeys to serve as known negative controls.

b. Infected monkeys: in order to cut across the clinical spectrum of the disease, weekly blood samples will be collected for assay of parasitemia, antigen, antibody and immune complexes, before onset of infection, early phase of infection, in overt disease, and following natural recovery. Very severely ill monkeys with a poor prognosis will be chemotherapeutically treated.

c. Naturally recovered monkeys: from section (b) above shall be monitored at 2 weekly intervals for parasitemia, antigen, antibody and immune complexes for a period of 12 weeks.

d. Chemotherapeutically cleared (cured): monkeys from section (b) above shall be monitored at 2 weekly intervals for parasitemia, antigen antibody and immune complexes for a period of 12 weeks.

4. Preparation of monospecific anti-*P. falciparum* antibody (autologous antibody)

The above *P. falciparum* strains will be propagated in cultures using homologous squirrel monkey erythrocytes and serum according to the previously described procedure (51) which is a modification of Trager and Jensen's method (52). Soluble antigens derived from the supernatant culture medium will be emulsified with Saponin (Quil-A) adjuvant. The emulsion will then be injected repeatedly into squirrel monkeys for production of hyperimmune serum. This method of antibody production was proven as a reliable means for production of monospecific anti-*P. falciparum* antibodies. When indicated the antiserum may be adsorbed with plasmodium-free monkey erythrocyte cultures, aliquoted and stored at -70° C.

5. Preparation of reference reagents

a. Culture-derived antigens: *P. falciparum* soluble antigens shall be prepared from cell culture supernatants as described elsewhere (31). These will be used for initial standardization of the test in the monkey model.

b. Plasma soluble circulating antigen(s): Sera (or plasma) from primary *P. falciparum* human infection, positive for circulating antigen will be used as a standard antigen.

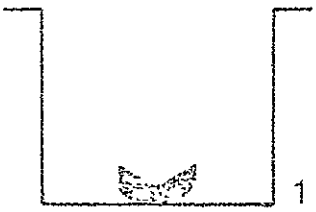
6. DOT-ELISA for detection of *P. falciparum* antigens and immune complexes

The principle of the system is diagramatically illustrated in figure 2 and is a modification of the technique of Singh and Tingle (33). Briefly, monospecific anti-*P. falciparum* IgG will be adsorbed onto nitrocellulose membrane. This solid phase serves as a "capture" mechanism for antigen in the test sample. After interaction with the test sample enzyme labelled-monospecific antibody is added. Subsequent addition of a precipitate enzyme substrate (4-chloro-1-Naphthol) leads to hydrolysis which is directly proportional to the amount of antigen present in the test sample. A blue dot develops on the membrane and the intensity of the dot depends on antigen concentration.

The C1q-binding assay (Fig. 3) will be based on the high affinity of immune complexes for C1q (33). Monospecific antibody

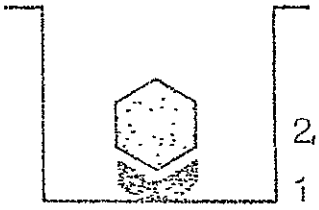
Fig. 2 Standard Microplate E L I S A for Soluble Circulating Ag (or Culture Derived *P. falciparum* Ag) [Ag "Capture" method]*

1 Monkey monospecific anti-*P falciparum* adsorbed to plate



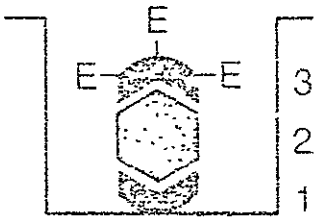
Wash PBS-Tween

2 Test sample containing antigen added



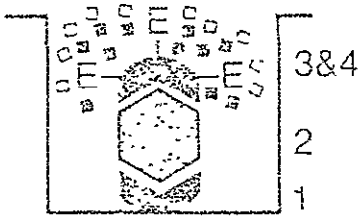
Wash PBS-Tween

3 Add enzyme labelled specific antibody



Wash PBS-Tween

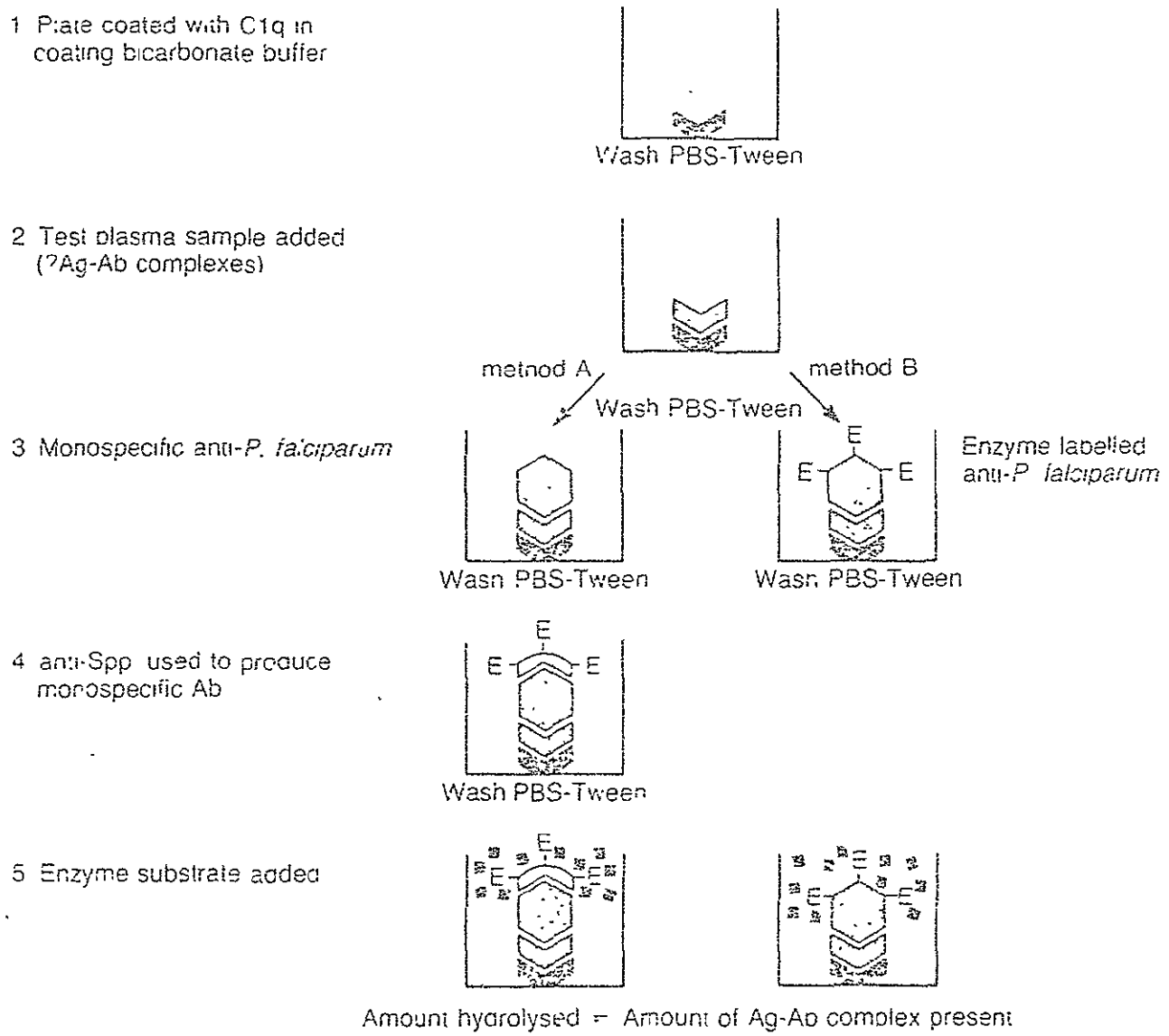
4 Add enzyme substrate



Amount hydrolysis = Amount antigen present

*Ag may also be directly adsorbed (coated) onto plate or nitrocellulose membrane in the DOT-ELISA

Fig 3 The C1q-binding Ag specific immunoassay for *P falciparum* depends on affinity of Ag-Ab complexes for C1q



labelled with peroxidase will then be used to locate *P. falciparum* antagen in the immune complex bound by Clq. After addition of substrate, the optical density of the hydrolysate corresponds to the quantity of soluble immune complexes in the test sample. Since a precipitating substrate (4-chloro-1-Napththol) is used, a blue dot represents a positive reaction. Positivity will be scored on a scale of 0-4 by reference and visual comparison with the standards giving a known degree of reaction.

7. Field studies in human populations in Colombia and Venezuela

In order to avoid statistical bias, randomely selected participants will include: a) "normal" - malaria-free individuals from high altitude areas known to be free from malaria; b) "suspects"-early cases (parasitologically negative); c) fulminating malaria cases; d) clinically confirmed naturally recovered cases; e) "carriers"...individuals who are clinically normal but with demonstrable *P. falciparum* in blood smears; f) chemotherapeutically "cured" individuals.

8. Collaborating scientists in Colombia and Venezuela

a. Colombia: Dr. Carlos Espinal, Director Malaria Vaccine Research, Bogota, Colombia, will coordinate the sample selection and collection from epidemiologically, clinically and parasitologically defined population clusters in Colombia.

A hospital in Villavicencio (malaria endemic area near Bogota) will be the focal point for the patients and field investigations.

b. Venezuela: Dr. Andres Sucre, Director, Malaria Control Program in Maracay, Venezuela will oversee patient selection and care in Venezuela along the same lines stipulated for Colombia.

9. Statistical methods

a. Experimental monkeys: All the animals will be healthy adult Bolivian Squirrel monkeys matched according to sex, age and statistically computed principal: control ratio. The health of the animals will be monitored daily (nutrition, etc.).

b. Organization and analysis of data. All data collected in the two major phases shall be analyzed for the specificity, sensitivity and predictive value of the ELISA test using the 3 x 3 table format (34).

The following formulas will be used: (following page)

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives} + \text{False negatives}} \times 100$$

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True negatives} + \text{False positives}} \times 100$$

$$\text{Predictive value} = \frac{Pa}{Pa + (1-p)\ (1-b)}$$

Where a = sensitivity, b = specificity

and P = prevalence

$$\text{Efficiency} = \frac{100 \times \text{True Positives} + \text{True Negatives}}{\text{Grand Total}}$$

In all cases the standard error (SE_p) of percentage shall be computed as follows:

$$SE_p = \frac{Pq}{n}$$

Where P = % sensitivity or specificity
q = 1-P; n = sample size

95% confidence limits = % $\pm$ 2·SE

VII. RESEARCH TIMETABLE AND DURATION

Phase I: Standardization of DOT-ELISA in experimental monkey model - 1½ years.

Phase II: Field evaluation of DOT-ELISA in Colombia and Venezuela - 1½ years.

VIII. REFERENCES

1. Quaky, I.A. (1980). Development and validation of an Enzyme Linked Immunosorbent Assay for Malaria. Tropenmed. Parasit. 31:325-333.

2. Kagan, I.G. (1972). Seroepidemiology and Serology of Malaria. Exp. Parasit. 31:126-135.

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Dr. James Erickson
Malaria Research Officer
Office of Health, Bureau for
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Agency for International Development
Washington, D.C. 20523

July 30, 1984

Dear Dr. Erickson,

Our company is pleased to submit for your consideration a research grant proposal entitled "Development of a simple Immunodiagnostic Test for Malaria suitable for performance in the Physician's office".

Your office sponsors major research efforts towards the development of malaria vaccines and similar studies are underway in many other laboratories throughout the world. However, neither vaccines nor chemotherapy can be rationally implemented by any medical team without adequate knowledge of the precise disease status of the respective patient and the population. Consequently, the need for differential specific identification of current malaria infection is an essential prerequisite to the effective implementation of control measures.

At the present time, most frequently used methods for diagnosis of malaria are limited to clinical observations and microscopic examination of stained blood films of respective patients. Such methods may be useful for the diagnosis of acute forms of the disease but their use is limited and usually ineffective for the diagnosis of subclinical and carrier forms of malaria. For this reason serologic methods are being used in an effort ascertaining the presence of the latter two forms of the disease. However, these antibody detection methods simply measure evidence of exposure to

the etiologic agent rather than provide an evidence of an ongoing infection. In most cases the presence of antibody cannot differentiate between current and previous infection. These methods, therefore, provide basically a presumptive diagnosis with a fundamental problem being that the measurable humoral immune response generally occurs long after initial exposure and persists beyond clinical cure.

While results of antibody detection tests are only indicators of the host's exposure to a given organism, detection of circulating parasite antigens is a strong evidence of an ongoing current infection. Based on studies of various erythrocytic protozoan infections, including malaria, there is a direct correlation between the level of parasitaemia and circulating soluble plasma "exoantigens". Free plasma exoantigens would thus be diagnostically detectable before, during and after peripheral blood parasitaemia is in evidence.

The proposed DOT-ELISA system will be the first diagnostic test suitable for the detection of actual plasmodial infection during both clinical and subclinical (latent) forms of the disease. In this test, large numbers of samples may be processed within a two hour period. The test can be carried out at room temperature and can be read visually. The reagents used in the DOT-ELISA are estimated to cost less than 5 cents per sample. The advantage of this procedure in comparison to the currently used parasitologic procedures for diagnosis of malaria are discussed in the enclosed research proposal.

This proposal is intended for internal review of the agency. The proposal contains highly proprietory material and must not be released as a public information to anybody.

Sincerely

Gottfried Kellermann PhD

President

Enclosures: 2 copies of proposal

B U D G E T

	Year 1	Year 2	Yea.
A. Salaries and Fringe Benefits	138,000	148,028	161,02[illegible]
2 person years - professional 3 person years - technicians 1 person year - consultant 0.5 person year - clerical			
B. Animals	5,000	5,500	5,000
Primates, Rabbits			
C. Equipment	6,000	6,600	5,500
Circulatory apparatus for antigen production			
D. Expendable supplies	18,500	17,650	18,000
E. Travel	4,500	4,900	4,000
Total Direct Costs	170,000	182,678	195,528
Total Indirect Costs (38%)	64,600	69,417	75,540
Total Costs	234,600	252,095	267,068