

PB-225 647

A MULTI-SPECIES MALARIA ANTIGEN FOR USE
IN THE INDIRECT FLUORESCENT ANTIBODY TEST

Alexander J. Sulzer, et al

Public Health Service

Prepared for:

Agency for International Development

1973

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

1. Report No. 614.532 54542		2.	
3. Title and Subtitle A MULTI-SPECIES MALARIA ANTIGEN FOR USE IN THE INDIRECT FLUORESCENT ANTIBODY TEST		5. Report Date 1973	
7. Author(s) Alexander J. Sulzer et al		8. Performing Organization Report No.	
9. Performing Organization Name and Address U.S. Public Health Service Center for Disease Control Atlanta, Georgia 30333		10. Project/Task/Work Unit No. 921-17-511-455	
		11. Contract/Grant No. PASA RA (IA) 5-68	
12. Sponsoring Organization Name and Address Department of State Agency for International Development Washington, D. C. 20523		13. Type of Report & Period Covered Research Bulletin	
14.			
15. Supplementary Notes			
16. Abstracts <p>A multi-species thick smear antigen containing equal proportions of <i>P. vivax</i>, <i>P. falciparum</i>, and <i>P. Brasiliense</i> schizonts was prepared for use in the indirect fluorescent antibody test for malaria. Tests with 80 sera showed that antibody titres with the multi-species antigen always equalled or exceeded the highest titre obtained when the sera were tested with the constituent mono-species antigens. Use of the multi-species antigen greatly reduces the time and labour required to screen serum specimens for malaria antibody. This study confirms the need for homologous antigens of all species to be detected if maximum sensitivity and reactivity are to be achieved.</p>			
17. Keywords and Document Analysis. 17a. Descriptors			
Reproduced by NATIONAL TECHNICAL INFORMATION SERVICE U.S. Department of Commerce Springfield VA 22151			
17b. Identifiers/Open-Ended Terms			
17c. COSATI Field/Group 614			
18. Availability Statement		19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 5
		20. Security Class (This Page) UNCLASSIFIED	22. Price \$3.00

614.532
120082

PASA RA(HA) 5-68

Reprinted from
TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.
Vol. 67. No. 1. pp. 55-58, 1973.

A MULTI-SPECIES MALARIA ANTIGEN FOR USE IN THE INDIRECT FLUORESCENT ANTIBODY TEST

ALEXANDER J. SULZER, MARIANNA WILSON, ALBERT TURNER AND
IRVING G. KAGAN

*Parasitology Section, Centre for Disease Control, Health Services and Mental Health
Administration, Public Health Service, U.S. Department of Health, Education and Welfare,
Atlanta, Georgia 30333*

For maximum sensitivity in the indirect fluorescent antibody (IFA) test for malaria, homologous antigens should be employed (SULZER, WILSON and HALL, 1969). In the comprehensive serological diagnosis of human malaria, therefore, each serum must be tested with the four human plasmodial species, a requirement which prolongs the time needed for the tests.

In this report the preparation and use of one single antigen, containing 3 malarial species, is described. The preparation technique is applicable to any combination of malaria parasites, or to *Plasmodium* in combination with other parasites. Use of this multi-species antigen in the malaria IFA test circumvents the need for multiple testing with several antigen species. The antigen should be, therefore, especially useful in screening large numbers of sera.

Materials and methods

Antigen was prepared by the washed-cell, thick-s smear technique described by SULZER and WILSON (1967). 3 malaria species, *Plasmodium vivax*, *P. falciparum* and *P. brasilianum*, were used. *P. brasilianum* has been shown to be serologically equivalent in the IFA test to *Plasmodium malariae* (COLLINS et al., 1966; COLLINS et al., 1967). After confirming this finding (unpublished data), we used it as a substitute for *P. malariae* antigen in this evaluation. *P. vivax* and *P. falciparum* were maintained in spleenectomized *Aotus trivergatus* monkeys and *P. brasilianum* in a splenectomized *Ateles geoffreyi* monkey. Infections were allowed to progress until at least 5% of the erythrocytes were parasitized. Since TARGETT (1970) reported that schizont antigens are more reactive than trophozoite antigens in the IFA test, a large ratio of schizonts to trophozoites were included in the antigen preparations. Since completely mature schizonts tend to rupture while the antigen is being prepared, the donor animals were bled when the parasites were at the 4-8 merozoite stage.

Immediately after the blood was withdrawn from the animal, the heparinized blood was centrifuged, the plasma removed, and the remaining plasma and soluble serum components eliminated by washing the cells 5 times in physiological phosphate buffered saline (PBS, pH 7.2). As previously reported, washed cells, with 1:10,000 merthiolate added to prevent bacterial growth, can be stored at 4 C. for at least 10 days with no loss of antigenic activity (SULZER et al., 1969). The washed cells, resuspended in PBS, were stored at 4 C. for 1 or 2 days until samples of all 3 species were available. Stained smears of the suspensions of each species were made. The number of parasites and the number of schizonts per field were recorded. The number of schizonts in the suspension with the fewest of these forms was taken as the base line; the suspensions of the other 2 species were diluted with PBS until their schizont count per field equalled the base line number. A count of not less than 15 schizonts per high power field was found desirable because of dilution in the final mix. After the number of schizonts in each suspension had been adjusted, equal volumes of the suspensions of each species were mixed, and the antigen slides were prepared.

This study was supported in part by the U.S. State Department, Agency for International Development, Participating Agency Service Agreement (PASA) No. RA (HA) 5-68.

Sera for evaluating the multi-species antigen were selected from specimens submitted to the Parasitology Section for routine malaria serology. Tests were performed with the multi-species antigen and with mono-species antigens of the 3 constituent species. Retrospective analysis of the 80 sera included in the battery showed 17 sera that did not react at the 1 : 16 dilution (the lowest endpoint dilution considered to be indicative of infection with malaria) and 63 sera with dilution titres ranging from 16 to 65,536 when tested with single species antigens. Most of the 80 sera specimens were from soldiers returned from South-east Asia, where *P. falciparum* and *P. vivax* are the dominant species. In the initial screening if the serum was reactive at dilutions of 1 : 16 and 1 : 64, titre determinations were made in coded tests; that is, test slides of both the mono-species and multi-species antigens were intermixed and coded before microscopic examination.

An American Optical Fluorescence Microscope,† equipped with Schott BG-12 and UG-2 exciter filters and a GG-9 barrier filter, was used in determining the reactions. After the slides were read, they were decoded and the results recorded. The dilution titres obtained with the multi-species antigen were compared with the highest dilution titres obtained with any of the mono-species antigens.

Results

The dilution titres of 80 sera tested with both multi-species and mono-species antigens are shown in Table I. With a single exception the maximum titre obtained with the 3 mono-species antigens and the titre obtained with the multi-species antigen did not differ by more than a factor of 4. In the single exception, the titre obtained with the multi-species antigen was 16 times the maximum mono-species titre. The multi-species antigen did not react with 5 sera that were positive by the mono-species antigens at the 1 : 16 dilution level; it reacted with 2, however, that were not positive with any of the 3 mono-species antigens.

Table II presents the results with 21 selected sera which had very low or no cross-reactions between *Plasmodium* species. The need for homologous antigens, especially

TABLE I. 80 sera grouped by serum dilution titres* using multi-species and single species antigens in the malaria IFA test.

	Serum dilution titre* using multi-species antigen						
	<16	16	64	256	1024	4096	16384
<16	17	2					
16	5	9					
64		3	7	4			
256			2	1	2	1	
1024				1	4	2	
4096					4	15	
16384							
65536							1

*Dilution factor of the endpoint dilution.

†Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

for diagnostic purposes, is graphically illustrated. 10 sera reacted with only 1 mono-species antigen; 11 others had very low cross-reactions with one or both of the other antigens. Most of the serum samples were from individuals infected with *P. falciparum* or *P. vivax*. If only *P. falciparum* antigen had been employed, 6 of the positive reactors would have been undetected; with only *P. vivax* antigen, 8 would have been missed. The higher number (13) were missed with the *P. brasilianum* antigen.

TABLE II. Serum specimens with dilution titres to only 1 of 3 mono-species malaria antigens or with very low cross-reactions.

Serum	Mono-species antigens			Titres with multi-species antigen
	<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. brasilianum</i>	
1	16*	Neg.	Neg.	16
2	Neg.	256	Neg.	1024
3	Neg.	64	Neg.	256
4	Neg.	16	Neg.	16
5	Neg.	16	Neg.	16
6	Neg.	16	Neg.	16
7	Neg.	16	Neg.	16
8	16	Neg.	Neg.	16
9	64	Neg.	Neg.	64
10	64	Neg.	Neg.	64
11	1024	16	16	4096
12	16	1024	16	1024
13	64	16	Neg.	64
14	16	64	Neg.	16
15	16	64	16	16
16	64	Neg.	16	64
17	16	256	16	4096
18	256	Neg.	16	64
19	Neg.	256	16	64
20	16	64	Neg.	256
21	Neg.	16	64	256

*Titre: dilution factor of the endpoint dilution.

Discussion

Several technical aspects are important in preparing a good antigen. Blood should be drawn from the donor with a parasitaemia of 5% or greater at a time when the ratio of schizonts to trophozoites is greatest. The higher the parasitaemia, the greater should be the dilution factor of the original blood volume; a high dilution factor helps eliminate non-specific background staining. Since each suspension serves as diluent for the 2 or 3 other batches of parasitized blood with which it is mixed, there should be 15-20 schizonts per high dry (40 \times) field in the mono-species suspensions to obtain at least 5 schizonts of each species in each field in the multi-species suspension.

Drawing parasitized blood with immature schizonts is very important. As noted, the washing manipulations may rupture mature schizonts. In addition, if the washed cells are stored for 1 to 2 days in the refrigerator, the parasites continue to develop slowly *in vitro*. If the freshly drawn blood contains mature schizonts, maturation *in vitro* may result in an antigen containing mostly free merozoites instead of schizonts in 1 or 2 days.

The reduction in time required for performing the malaria IFA test with a multi-species antigen instead of mono-species antigens is the greatest benefit derived from the

new antigen. In our laboratory, sera submitted for routine malaria serology are screened with antigens prepared from each of the 3 species. With the multi-species antigen, the time required for the screening step has been reduced by two-thirds. Antibody titrations can be made with the multi-species antigen if species identification is not required.

The multi-species antigen promises to be particularly advantageous when the IFA test is used to test large numbers of sera. The time required for titrating specimens in epidemiological surveys is almost prohibitive when, for maximum sensitivity, mono-species antigens of all species are employed. Surveys in which titrations are performed with only 1 human or simian *Plasmodium* species for antigen may have reduced sensitivity, especially for a malaria species that does not cross-react with the antigen employed in the test. With the multi-species antigen, test sensitivity for any one of the species employed is not lost, and the time needed to process samples is equivalent to that required for a test with 1 antigen only.

Summary

A multi-species thick smear antigen containing equal proportions of *P. vivax*, *P. falciparum*, and *P. brasilianum* schizonts was prepared for use in the indirect fluorescent antibody test for malaria. Tests with 80 sera showed that antibody titres with the multi-species antigen always equalled or exceeded the highest titre obtained when the sera were tested with the constituent mono-species antigens. Use of the multi-species antigen greatly reduces the time and labour required to screen serum specimens for malaria antibody. This study confirms the need for homologous antigens of all species to be detected if maximum sensitivity and reactivity are to be achieved.

REFERENCES

- COLLINS, W. E., JEFFERY, G. M., GUINN, E. A. & SKINNER, J. C. (1966). *Am. J. trop. Med. Hyg.*, **15**, 11.
———, SKINNER, J. C. & COIFMAN, R. E. (1967). *Ibid.*, **16**, 568.
SULZER, A. J. & WILSON, M. (1967). *J. Parasit.*, **53**, 1110.
———, ——— & HALL, E. C. (1969). *Am. J. trop. Med. Hyg.*, **18**, 199.
TARGETT, G. A. T. (1970). *Clin. exp. Immunol.*, **7**, 501.