ELISA method for detecting *Plasmodium falciparum* circumsporozoite antibody*

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An enzyme-linked immunosorbent assay (ELISA) for circulating IgG mouse antibody to Plasmodium falciparum *circumsporozoite (CS) protein was modified for use with human sera collected in an area of northern Zambia that was endemic for malaria and from individuals never exposed to malaria. Optimum sensitivity was achieved using Immulon 2 microtitration plates, boiled casein–Tween 20 blocking buffer, and by adding a solution of boiled casein (4 µg/ml) to the capture antigen diluent. The results for the detection of anti-CS IgG correlated well with those of sporozoite immunofluorescence antibody assays. Modification of the ELISA method permitted the simultaneous detection of anti-CS IgG and IgM antibody on a single serum sample in the same well of the microtitration plate and the detection of anti-CS IgG antibody in Kenyan dried whole-blood samples collected on filter-paper. The assay has been used to monitor human antibody levels in a phase-I malaria vaccine trial and in longitudinal studies of malaria transmission in Thailand and Kenya.*

Introduction

The sequencing and production of the circumsporozoite (CS) protein of *Plasmodium falciparum* has permitted the development of candidate malaria vaccines based on synthetic peptides (2, 18) and recombinant proteins (17). Immunoradiometric (IRMA) (19) and enzyme-linked immunosorbent assays (ELISA) (5,6) have been developed to detect and monitor antibodies produced against CS antigens. Such assays are quantitative and more sensitive than the immunofluorescence antibody (IFA) assay for the detection of anti-CS antibody (10–12). IRMAs and ELISAs may be useful also in elucidating the role played by naturally induced antisporozoite antibodies in the epidemiology of malaria (14).

We have modified an ELISA method originally developed to detect *P. falciparum* CS antibody in mouse sera (17) and have tested it against sera collected in an area of northern Zambia that is endemic for malaria. However, excessive background absorbance, i.e., in the absence of capture antigen, was observed with some of the sera. To reduce background absorbance, without reducing the test sensitivity, we varied the following: the type of microtitration plate, the capture antigen, the blocking solution, the procedure used to pretreat the test serum, the enzyme-labelled antihuman IgG antibody, and the substrate solution. A modification of this ELISA procedure that uses peroxidase- and phosphatase-conjugated reagents permits simultaneous detection of both anti-CS IgG and IgM antibodies on a single serum sample in the same microtitration plate well. Furthermore, to address the difficulties associated with collecting and transporting refrigerated or frozen samples, we evaluated the method using whole blood samples that had been dried on filter-paper, stored at ambient temperature, and eluted before analysis for anti-CS IgG antibody. The results of these investigations are reported here.

Materials and methods

Microtitration plates

The following types of plates were tested in pilot assays: Falcon;[a] Dynatech flexible[b] and Immulon 1 and 2;[b] Costar;[c] and Linbro.[d] The Falcon and

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Dynatech plates were made of flexible polyvinyl chloride, and the others of rigid polystyrene. Only the central block of wells was used to discount any "edge" effect, whereby on some plates a substrate colour change is associated with the peripheral wells (9).

Capture antigens

Two capture antigens were tested: R32tet$_{32}$ and R32LR. The R32tet$_{32}$ (R32) was a purified *P. falciparum* CS recombinant protein construct that contained 30 Asn-Ala-Asn-Pro and two Asn-Val-Asp-Pro tetrapeptide repeats (a total of 128 amino acids) that were fused to a 32-amino-acid tail derived from the tet^r region of the PAS1 plasmid (17). The R32LR capture antigen was identical to the R32, except that it contained only the first two amino acids (Leu-Arg) of the 32-amino-acid fusion tail (13). Only selected sera were tested against the R32LR capture antigen.

Test wells, which contained capture antigen in diluent, and control wells, which contained diluent only, were run concurrently in adjacent columns to permit subtraction of background absorbance caused by non-antigen-antibody binding. Diluents for the capture antigens included Dulbecco's phosphate-buffered saline (PBS), pH 7.4,[e] and PBS containing various amounts of boiled casein.[f] Boiled casein was prepared by suspending 5.0 g of casein in 100 ml of 0.1 mol/l sodium hydroxide and then bringing this to the boil. After the casein had dissolved, 900 ml of PBS was added, the solution was allowed to cool, the pH adjusted to 7.4 with hydrochloric acid, and 0.1 g of thimerosal[e] and 0.02 g of phenol red[e] were added.

Blocking buffer

In addition to a standard blocking buffer (a mixture of 1.0% bovine serum albumin (BSA), 0.5% casein, 0.005% thimerosal, and 0.001% phenol red in PBS) (16), the following blocking solutions were tested: 0.5% and 2% solutions of BSA;[e] boiled casein or nonfat dried milk,[g] with and without the addition of 1.0% polyoxyethylenesorbitan monolaurate (Tween 20);[e] or 10% goat, rabbit, or porcine serum. Blocking solutions were kept refrigerated and fresh solutions were made weekly.

Treatment of sera and collection on filter-paper

The sera used for ELISA development were collected from 130 individuals aged 4–71 years who lived in an area of northern Zambia that was endemic for *P.*

falciparum malaria. Selected Zambian sera with high background absorbances were centrifuged (5000 *g*, 20 minutes, 4 °C) or filtered under sterile conditions (0.2-μm cellulose acetate microfilter).[h]

Sera collected from August 1986 to January 1987 in an area of Thailand where there was active transmission of malaria (14) were used to detect IgG and IgM antibodies in single microtitration plate wells, and to determine the linearity of absorbance values using serially diluted sera.

Blood for evaluating the filter-paper method was prescreened using samples collected between 16 January and 6 February 1986 from 61 field and laboratory technicians from the Kisumu region of Kenya, where *P. falciparum* is holoendemic (3). Sera were tested for anti-CS IgG antibody using the ELISA method; absorbance values ranged from negative (0.00) to > 2.0 (1-hour's reaction time; $\lambda = 414$ nm). Venous blood samples were drawn on 5–6 March 1986 from ten individuals from this group and processed as follows: the serum was separated, transported on wet ice for 48 hours, and then stored frozen at −70 °C; whole blood collected in tubes that had been treated with ethylenediaminetetraacetic acid (EDTA) was kept sterile, transported as described for the serum, and then stored at 4 °C; and whole blood samples were immediately transferred to filter-paper[i] (8) using a micropipette and disposable polypropylene tips (50 μl per spot), air dried, placed in slide boxes, and transported and stored at ambient temperature and humidity. After 5 days, the areas on the filter-papers with the dried samples were cut out, placed in capped tubes, and eluted in 1.0 ml boiled casein blocking buffer containing 0.1% Tween 20 (1:20 dilution, 12 hours, 4 °C). Samples were tested at 1:20 and 1:100 dilutions for anti-CS antibody using the ELISA procedure.

Anti-human enzyme-labelled antibodies

Peroxidase-linked anti-human IgG (H + L) antibodies (produced in goats), obtained from Miles-Yeda[j] and Kirkegaard & Perry Laboratories,[k] and phosphatase-linked goat anti-human IgM (μ-chain specific) antibodies, also obtained from Kirkegaard & Perry, were tested. The lyophilized antibodies obtained from Kirkegaard & Perry were dissolved in a 1:1-v/v mixture of distilled water and glycerin to give a concentration of 0.5 mg/ml.

Substrate solutions

The following solutions of 2,2'-azinobis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) peroxidase substrate were tested: a premixed commercial preparation obtained from Kirkegaard & Perry, a mixture of 1 mg ABTS (Sigma Chemical Co.) per ml of 0.1 mol/l citrate-phosphate buffer (pH 4.0) to which was added 10 μl of 1% (v/v) hydrogen peroxide immediately before use. The phosphatase conjugate was used with either a *p*-nitrophenyl phosphate substrate kit obtained from Kirkegaard & Perry or a mixture of 1 mg disodium *p*-nitrophenyl phosphate (Sigma Chemical Co.) per ml of kit buffer.

ELISA method

An ELISA method developed to monitor CS antibody produced against *P. falciparum* candidate vaccines in mice (17) was modified for use with human sera. All tests were conducted at room temperature. Into each well of the microtitration plate was pipetted 50 μl of the screening antigen solution (2 μg R32 per ml PBS), and the plate was then covered and stored overnight. Approximately 18 hours later, the well contents were aspirated, the wells then filled with blocking buffer, and set aside for 1 hour. Test sera were diluted in blocking buffer, 50 μl of the resultant solution was added to each well, and the plate covered. After a 2-hour incubation, the well contents were aspirated, the wells washed three times with a 0.5% (v/v)-solution of Tween 20 in PBS (PBS–Tw), and 50 μl of peroxidase-conjugated anti-human IgG diluted with blocking buffer was added to each well. For the combined detection of IgG and IgM, the assay was modified by adding phosphatase anti-human IgM to the peroxidase anti-IgG solution. After 1 hour, the well contents were aspirated, the wells washed three times with PBS–Tw, and 100 μl of ABTS substrate was added to each well. The absorbance at $\lambda = 414$ nm was determined at the designated times using an ELISA plate reader.[/] In the combined assay for IgG and IgM, the peroxidase substrate was then aspirated, the wells were washed as described above, 100 μl of phosphatase substrate was added, and the absorbance at $\lambda = 414$ nm recorded.

Each serum was assayed in triplicate at a 1:100 dilution. The mean absorbance of three wells that contained diluent without capture antigen was subtracted from the absorbances of three adjacent wells that contained capture antigen, and the mean and standard deviation (SD) were calculated. This format permitted 10 sera to be assayed per plate without using the peripheral wells. Selected sera also were diluted serially and assayed.

[/] Titertek Multiskan. Flow Laboratories, Inc., McLean, VA, USA.

Immunofluorescence antibody assays

The IFA assays were conducted using air-dried salivary gland sporozoites (17). Fluorescence levels were graded from zero to 4+, with zero corresponding to background fluorescence, and 4+ to intense uniform fluorescence over the entire sporozoite. Only selected sera were tested by IFA.
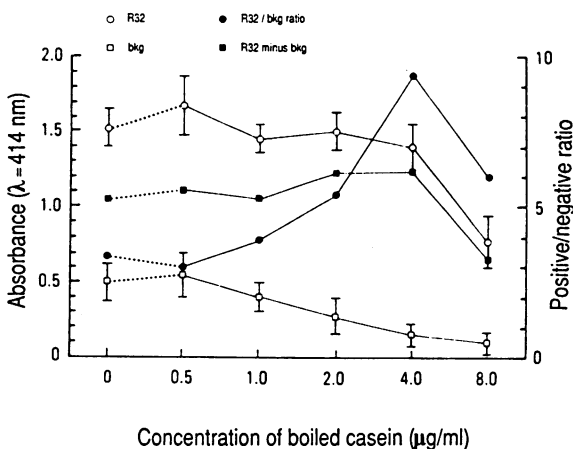
Results

Microtitration plates

Initial results indicated that the Immulon 2 plates consistently gave lower background (bkg) (PBS only) values, higher positive minus background values (R32−bkg), and higher positive to background (R32/bkg) ratios (Table 1). Lower positive (R32−bkg) or higher background (PBS only) absorbances were observed with the other microtitration plates. The Immulon 2 plates were used for all the additional tests.

Use of 150 μl of screening antigen solution per well resulted in proportionally higher background values without increasing the sensitivity, and 50 μl of the antigen solution was therefore used in subsequent tests.

Fig. 1. **Plots of the absorbance values obtained in the ELISA for anti-*Plasmodium falciparum* circumsporozoite IgG antibody with R32 capture antigen and of the positive to negative ratios for serum that contained absorbances with various amounts of boiled casein in the capture antigen diluent (bkg = background). Absorbances were measured at $\lambda = 414$ nm, after 1-hour's reaction time.**

Concentration of boiled casein (μg/ml)

Table 1: **Comparison of the absorbance values obtained with various types of microtitration plates in the ELISA method to detect anti-*Plasmodium falciparum* circumsporozoite human IgG antibody (unless indicated, plates were made of rigid polystyrene)**

Serum reference number	Capture antigen	Absorbance value[a]					
		Falcon[b]	Dynatech[b]	Immulon 1	Immulon 2	Costar	Linbro
288	R32	1.21	1.12	0.85	1.05	0.89	0.78
	PBS	0.43	0.32	0.43	0.25	0.42	0.45
	R32 − PBS	0.78	0.80	0.42	0.80	0.47	0.33
	R32/PBS ratio[c]	2.81 (A)	3.50 (B)	1.98 (C)	4.20 (D)	2.12 (C)	1.73 (C)
364	R32	1.88	>2.00	1.43	>2.00	>2.00	1.89
	PBS	1.29	1.02	0.76	0.75	0.83	0.94
	R32 − PBS	0.59	>0.98	0.67	>1.25	>1.17	0.95
	R32/PBS ratio[c]	1.46 (E)	>1.96 (F)	1.88 (F)	>2.67 (G)	>2.41 (G)	2.01 (F)

[a] Mean of triplicate samples, all SD ⩽ 10% of mean; $\lambda = 414$ nm; at 1-hour's substrate reaction time.
[b] Made of polyvinyl chloride.
[c] Ratios followed by a different letter in parentheses were significantly different from each other (χ^2 test, $P = 0.05$).

Capture antigen and diluents

Addition of boiled casein to the CS capture antigen PBS diluent reduced the background absorbance and increased the R32/bkg ratio (Fig. 1). A capture antigen diluent concentration of 4 μg boiled casein per ml PBS was selected for routine use since it reduced background absorbance values, usually to below 0.2 after 1-hour's reaction time, but maintained high absorbance values for positive sera (Fig. 1 and 2).

Selected sera that were positive for R32 exhibited no significant reduction in absorbance values ($P > 0.05$, Student's t-test) when R32LR was used as the capture antigen.

Blocking buffer

Boiled casein and BSA were more effective blocking solutions than powdered milk. Use of 2% solutions was no more effective than of 0.5% for BSA or boiled casein; however, the addition of 1% Tween 20 consistently produced lower background values and higher R32/bkg ratios. The addition of 10% heterologous serum to the blocking solution or enzyme diluent had no detectable effect on sensitivity or reduction of background absorbance. The most effective blocking buffer tested consisted of boiled casein (0.5%) containing 1% Tween 20.

## Treatment of sera and collection on filter-paper

Centrifugation or sterile filtration of selected sera did not consistently reduce background absorbance values. The use of a solution of 0.025% Tween 20 in 0.5% boiled casein reduced nonspecific binding, since background values were consistently lower than those in concurrently tested wells without Tween 20 in the serum diluent.

Fig. 2. **Absorbance values obtained in the ELISA for anti-*Plasmodium falciparum* circumsporozoite IgG antibody for six sera using various amounts of boiled casein in the capture antigen diluent. Absorbances were measured at $\lambda = 414$ nm after 1-hour's reaction time. See text for IFA data on the sera.**
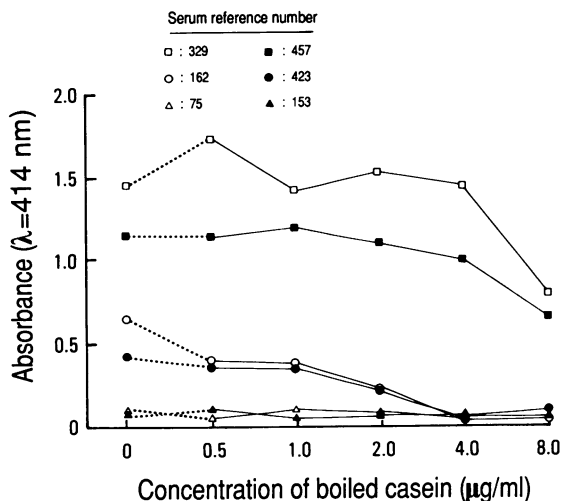
Excellent sensitivity and R32/bkg ratios were obtained with 1:100 dilutions of sera and a 2-hour incubation time. The proportion of Zambian test subjects who were positive for CS antibody together with their ages are shown in Fig. 3. The ELISAs obtained for serial dilutions of five Thai sera gave linear dose–response plots with correlation coefficients (r) ranging from 0.82 to 0.99 for dilutions with

Fig. 3. **Distribution by age of Zambian donors who were positive in the ELISA for anti-*Plasmodium falciparum* circumsporozoite IgG antibody. The sample size is shown above each bar in the plot.**
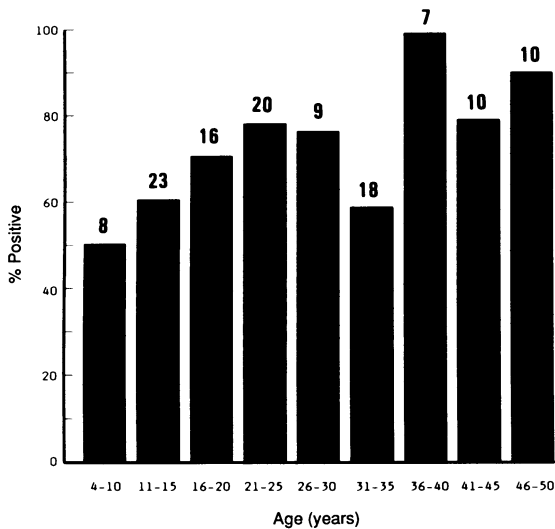
absorbance values of 0.10–1.90. The results for the five Thai sera tested for anti-CS IgG and IgM antibody both in separate wells and when combined in a single well are shown in Fig. 4. The absorbance values obtained when these sera were tested for both IgG and

Fig. 4. **Results of the ELISA for anti-*Plasmodium falciparum* circumsporozoite IgG and IgM antibodies determined in either separate microtitration plate wells or by a combined assay in the same well. Sera 129a and 129b were collected on 16 August 1986 and 4 January 1987, respectively. Absorbances were measured at $\lambda = 414$ nm after 1-hour's reaction time.**
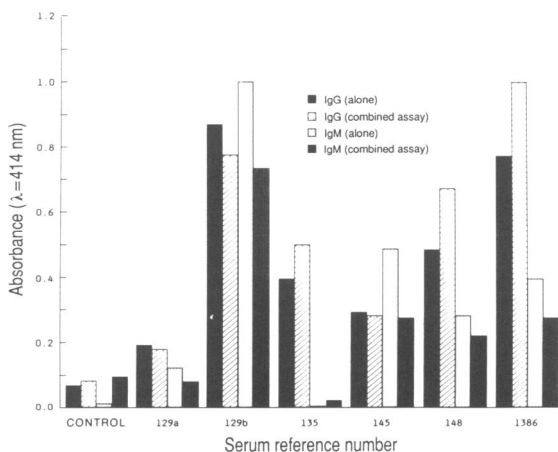
IgM antibodies in the same well differed by $+14.5\%$ and -27.1%, respectively, from the values obtained in separate wells.

The results obtained with the ELISA for serum, uncoagulated EDTA-treated whole blood, and dried whole blood eluted from filter-papers are given in Table 2. Included also is the combined mean $\pm$ standard deviation for the background absorbance (wells without R32 capture antigen) for 1:20 and 1:100 dilutions for all 10 test sera.

Anti-human enzyme-labelled antibodies

Only minor differences were observed in absolute sensitivity of the ELISA for the peroxidase-conjugated anti-IgG antibodies obtained from different commercial sources. Optimum working dilutions of stock solutions ranged from 1:100 (for antibodies from Kirkegaard & Perry) to 1:2000 (for antibodies from Miles–Yeda). The optimum dilution of the phosphatase-linked anti-IgM antibody was 1:200.

Substrate solutions

The peroxidase substrate prepared by dissolving ABTS (obtained from Sigma Chemical Co.) in colourless citrate-phosphate buffer produced a slightly coloured solution with an absorbance of approximately 0.1. Commercial substrate prepared by mixing equal volumes of ABTS and hydrogen peroxide solution produced a colourless substrate (absorbance $\leqslant 0.02$).

Phosphatase substrate prepared according to the directions supplied with the kit also gave a slightly coloured solution with an absorbance of approximately 0.1. Use of disodium *p*-nitrophenyl phosphate (obtained from Sigma Chemical Co.) with the buffer kit produced a colourless substrate solution (absorbance $\leqslant 0.02$). Absorbance readings ($\lambda = 414$ nm) were made on freshly mixed solutions after blank readings had been taken on empty wells.

Immunofluorescence antibody assays

There was excellent agreement between the activities determined by IFA and ELISA for the sera tested. Ten ELISA-negative sera (absorbance < 0.2) were also negative by IFA; 10 sera with intermediate ELISA values (absorbance $= 0.5$–1.0) gave IFA results that ranged from $1+$ to $3+$; and 10 sera that exhibited high ELISA activity (absorbance > 1.5) had IFA values of $4+$. Sera 75, 153, 162, and 423 were negative by IFA, while sera 329 and 457 were $3+$ and $2+$, respectively (Fig. 2).

Discussion

The ELISAs and IRMAs that we have described could play an important role in evaluating CS-antigen-based

Table 2: **Results of the ELISA method for the detection of anti-*Plasmodium falciparum* circumsporozoite IgG antibody in samples of human serum, whole blood, and eluted dried whole blood samples**

Reference number of subject	Dilution	Absorbance value[a]		
		Serum	Whole blood	Dried whole blood
27	1:20	1.25 ± 0.03	1.27 ± 0.19	1.67 ± 0.04
	1:100	1.62 ± 0.13	0.46 ± 0.05	0.57 ± 0.04
34	1:20	>2	>2	>2
	1:100	>2	0.53 ± 0.05	0.45 ± 0.03
49	1:20	>2	>2	>2
	1:100	1.00 ± 0.09	0.62 ± 0.02	0.60 ± 0.05
51	1:20	0.25 ± 0.07	0.02 ± 0.01	0.07 ± 0.02
	1:100	0.06 ± 0.02	0.00	0.00
52	1:20	1.23 ± 0.03	0.29 ± 0.04	0.43 ± 0.03
	1:100	0.37 ± 0.02	0.08 ± 0.02	0.11 ± 0.01
55	1:20	0.26 ± 0.08	0.11 ± 0.04	0.06 ± 0.04
	1:100	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
56	1:20	>2	0.64 ± 0.07	0.62 ± 0.13
	1:100	0.26 ± 0.03	0.13 ± 0.01	0.16 ± 0.04
60	1:20	0.72 ± 0.05	0.27 ± 0.02	0.26 ± 0.03
	1:100	0.15 ± 0.01	0.05 ± 0.01	0.06 ± 0.02
65	1:20	1.02 ± 0.06	0.55 ± 0.07	0.42 ± 0.02
	1:100	0.23 ± 0.01	0.09 ± 0.02	0.05 ± 0.00
66	1:20	>2	0.90 ± 0.08	0.93 ± 0.06
	1:100	0.60 ± 0.03	0.24 ± 0.03	0.20 ± 0.02
Combined mean $\pm$ SD background absorbance values[b]	1:20	0.16 ± 0.05	0.17 ± 0.04	0.21 ± 0.06
	1:100	0.07 ± 0.03	0.06 ± 0.02	0.06 ± 0.03

[a] Shown are the mean $\pm$ SD from three wells containing R32 capture antigen, after subtraction of background absorbance; $\lambda = 414$ nm; 1-hour's substrate reaction time.
[b] For all 10 sera tested.

vaccines as well as in elucidating the function of anti-sporozoite antibody in the epidemiology of malaria. In the ELISA the sensitivity for anti-*P. falciparum* CS protein was optimized and the background absorbance reduced by an appropriate choice of reagents and microtitration plate. The ability of the method to test for both IgG and IgM antibodies using a single sample of serum in the same microtitration plate well reduces assay time and the amounts of test serum and reagents required. Also, since anti-CS IgG antibody can be eluted from blood samples dried on filter-paper and transported at ambient temperature, use of the assay can be extended to areas where sample refrigeration is not feasible.

For the ELISA the most effective method for reducing the high background absorbance observed with some sera, while maintaining the sensitivity, was to add boiled casein solution (4 µg/ml) to the capture antigen diluent. The reason for this is not clear, however, since use of a solution of 0.5% boiled casein

in the 1-hour blocking step immediately after absorption of the capture antigen did not reduce excessive background absorbance as effectively.

Both boiled casein and BSA proved to be effective blocking agents in the ELISA; however, casein is considerably cheaper and use of it substantially reduces the cost of the assay. Use of casein from sources other than J.T. Baker or of other grades of BSA usually resulted in increased background absorbance values or reduced positive to negative ratios. Addition of 1% Tween 20 to the blocking solutions also reduced background absorbance values. Subsequent testing demonstrated that 0.5% boiled casein that contained 0.1% Tween could be used for both blocking and serum dilution without decreasing the sensitivity or increasing the background absorbance. The use of 10% heterologous serum to block or dilute the samples or of heating, centrifugation, or sterile filtration did not reduce consistently high background absorbance.

For the ELISA, peroxidase anti-human IgG conjugate was chosen in preference to phosphatase-linked anti-human IgM because of its more rapid colour development. Both the commercially obtained anti-human IgG peroxidase conjugates that were tested functioned well, but that obtained from Miles–Yeda was selected for routine use because the optimum sensitivity and low background absorbance were achieved at a 1:2000 dilution, making it the more economical.

When using a plate reader, blanking on microtitration plate wells that contain substrate results in automatic subtraction of substrate absorbance. However, visual determinations were easier and more accurate with the clear substrate solutions. The commercial peroxidase substrate was more convenient to use, but it is more expensive and requires to be refrigerated for long-term storage.

Comparison of the five types of microtitration plate indicated that the Immulon 2 (with "U"-shaped wells) was the most satisfactory for ELISA development and the assay was therefore optimized for this plate. The method functioned equally satisfactorily with plates having either "U"-shaped or flat-bottomed wells; however, the "U"-shaped wells were more convenient to wash and easier to read visually. No difference was detected between the two shapes of well when the plate reader was used.

There was no significant reduction in absorbance values ($P > 0.05$, Student's t-test) when R32LR was used against selected sera. The antibody detected was therefore active against the CS portion of the R32 and not the fusion tail protein (*17*). This conclusion is also supported by the excellent agreement between the data obtained in the sporozoite IFA and the ELISA with the R32 capture antigen.

The proportion of anti-CS IgG antibody in the Zambian sera increased with the donor's age for 4–25-year-olds. This is in agreement with other studies that have examined donor age versus the level of sporozoite antibody in sera collected from areas where *P. falciparum* is prevalent (*4–6, 10–12*).

Testing for both anti-CS IgG and IgM on a single sample of serum in the same microtitration plate well reduces considerably the amount of supplies, reagents and sample required, as well as the total assay time. However, the reduced sensitivity to IgM that results may mean that this modification should be used only for general screening purposes.

Excellent correlation was observed between the absorbance values for blood samples and eluted dried blood. However, the absorbance values for samples of whole blood, either EDTA-uncoagulated or dried and eluted from filter-paper, were consistently lower than those for sera. Also, some samples that were weakly positive when sera were used became negative when retested using whole blood. If refrigeration is not available, measured volumes of sera can be dried on filter-paper for later elution and testing.

Storage and shipment of lyophilized peroxidase anti-human IgG antibodies and the R32 capture antigen at ambient temperatures had no detectable effect on the sensitivity of the assay, and greatly facilitated distribution of reagents.

The ELISA method described has been used to monitor antibody levels in a human phase-I safety and immunogenicity study of a recombinant *P. falciparum* sporozoite vaccine (*1*) and in longitudinal studies of malaria transmission in Kenya (*7*) and Thailand (*14*). It has also been modified successfully to detect *P. falciparum* and *P. vivax* CS antibody using synthetic peptides conjugated to carrier proteins and *P. vivax* antibody using a recombinant protein.

Résumé

Méthode ELISA pour la détection des anticorps circumsporozoïtaires de *Plasmodium falciparum*

Une méthode immuno-enzymatique (ELISA) a été mise au point pour la détection des anticorps IgG circulants dirigés contre la protéine circumsporozoïtaire (CS) de *Plasmodium falciparum*. Pour cela, des sérums humains ont été recueillis dans une région du nord de la Zambie où le paludisme est endémique et chez des individus qui n'avaient jamais été exposés à la maladie. Différents types de plaques de microtitrage, d'antigènes CS, de diluants, de solutions tampons d'arrêt, de conjugués enzymatiques anti-homme, de substrats et de méthodes de traitement du sérum ont été évalués pour tenter d'améliorer la sensibilité et de réduire

l'absorbance de base (c'est-à-dire en l'absence d'antigène) observée avec certains sérums. Le type des plaques de microtitrage utilisées et l'origine des réactifs ont eu une influence significative sur la sensibilité et l'absorbance de base. La sensibilité optimale a été obtenue avec des plaques Immulon 2, en utilisant un mélange de caséine porté à l'ébullition et de Tween 20 comme solution d'arrêt, et en ajoutant au milieu de dilution de l'antigène une solution de caséine portée à l'ébullition (4 mg/l), un conjugué anticorps anti-homme-peroxydase et un substrat du commerce. L'antigène R32tet32 utilisé était une protéine CS de *P. falciparum* purifiée obtenue par génie génétique et contenant 30 unités tétrapeptidiques Asn-Ala-Asn-Pro et deux unités Asn-Val-Asp-Pro (soit 128 acides aminés au total) avec une queue de fusion composée de 32 acides aminés. Cela a permis de réduire notablement l'absorbance de base (de plus des deux tiers pour certains sérums). Pour tous les témoins négatifs, l'absorbance à 414 nm après un temps de réaction d'une heure a été inférieure à 0,2, tandis que 25% des sérums recueillis en Zambie présentaient une absorbance moyenne à forte ($\geqslant$ 0,5–2,0). Une bonne corrélation a été observée entre la détection des IgG anti-CS par l'ELISA et les résultats du dosage des anticorps antisporozoïtes par immuno-fluorescence. La proportion d'IgG anti-CS dans les sérums recueillis en Zambie augmentait avec l'âge des donneurs, de 4 à 25 ans. Des dilutions en série pratiquées sur les sérums recueillis en Thaïlande ont montré que la courbe dose-réponse de l'ELISA était linéaire. La méthode a été modifiée pour détecter: *a*) en Thaïlande, la présence d'IgG et IgM anti-CS sur un échantillon de sérum unique et dans la même cupule de microtitrage en utilisant des réactifs conjugués respectivement à la peroxydase et à la phophatase; *b*) au Kenya, la présence d'IgG anti-CS dans des échantillons de sang complet recueillis sur papier filtre et desséchés. En l'absence de moyens de réfrigération, les échantillons peuvent être conservés sur papier filtre après dessiccation, puis élués en vue du dosage. Cette méthode a été utilisée pour suivre l'évolution des taux d'anticorps humains lors des essais de phase I d'un vaccin contre le paludisme et dans des études longitudinales sur la transmission de la maladie en Thaïlande et au Kenya. Les réactifs nécessaires peuvent être lyophilisés et expédiés à la température ambiante, ce qui représente un atout considérable pour la diffusion de la méthode.

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