ANTIBODY DETECTION ELISAS FOR MALARIA DIAGNOSIS

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Abstract. Parasite extracts of *Plasmodium falciparum* and *P. chabaudi* and three synthetic peptides from the *P. falciparum* MSA2 merozoite antigen were tested for suitability as antigens in an antibody detection ELISA using sera from malaria patients in Brisbane. The *P. chabaudi* extract was superior to *P. falciparum* extract for detecting *P. vivax* cases, while for *P. falciparum* cases the two parasite extracts were equivalent. Single peptide antigens were generally less sensitive than parasite extracts; however, peptides G3 and G7 were more sensitive than parasite extracts in detecting first attacks of *P. vivax*. Examination of isotype specific responses demonstrated that this may be explained by higher IgG responses to these peptides in first than in subsequent *P. vivax* attacks. Because of the differing antibody specificities in primary and secondary *P. falciparum* and *P. vivax* cases, the best sensitivity was achieved by using the combined results of assays with three antigens: *P. chabaudi*, peptide G3 and peptide G7. The combined sensitivity was 77.1% for *P. falciparum* and 88.6% for *P. vivax* acute cases with 91.1% specificity.

INTRODUCTION

The current standard diagnostic test for malaria is examination of thick and thin blood films. This test is capable of detecting as few as 10 parasites per µl, but is highly labor-intensive as well as requiring trained and motivated personnel and carefully maintained equipment. The difficulties of extending these facilities so that immediate diagnosis is available at the periphery of the health systems in endemic countries has led to the search for new tests which will be fast, simple, reliable, cheap and suitable for batch processing of samples (WHO, 1988; Tharavanij, 1990).

In the clinical situation, requirements for a malaria diagnostic test vary according to the epidemiologic circumstances. The spectrum ranges from the highly endemic area with poor laboratory facilities to the non-endemic area with the best of modern facilities. Australia is in the latter category but is in close proximity to highly endemic countries, such as Papua New Guinea, Solomon Islands. In highly endemic areas, where fever has a strong likelihood of being caused by malaria, presumptive treatment is often given without laboratory confirmation of the diagnosis. In such circumstances, high levels of immunity may mean that low parasitemias are not necessarily of clinical importance, whereas detection of high parasitemias (*eg* in young children or in drug resistant cases) is vital to prevent severe morbidity or mortality. In non-endemic areas, on the other hand, malaria may not be the most likely cause of fever. Persons with no previous exposure to malaria may have symptoms when parasites are still at extremely low densities and thus hard to detect on a blood film. Confirmation of the diagnosis in these cases is important because of the possible rapid development of severe consequences, especially in the case of *P. falciparum* infections.

Diagnostic tests are also required for monitoring of malaria transmission during malaria control programs, and for screening potential blood donors to prevent accidental malaria transmission. In these circumstances speed and simplicity are not the major requirements of a test, since it will probably be performed at a central laboratory: here the suitability of a test for batch processing of samples and automation become more important.

Currently there are a number of diagnostic tests under evaluation which will be briefly reviewed.

Nucleic acid probes

Several groups have developed plasmid DNA or oligonucleotide probes, based on a 21-base pair repeat which constitutes up to 10% of the genome, for detecting *P. falciparum* (reviewed by WHO,

1986; Barker, 1990). Radiolabeled probes have achieved sensitivities of 81-89% and specificities of 94-97% (Mucenski *et al,* 1986; Barker *et al,* 1989 a, b) when tested on patients with suspected malaria in endemic areas. They are as sensitive as 'routine' microscopy in these cases, although 'expert' microscopists are superior. The probes are generally weaker in detecting low parasitemias such as those encountered in the early stages of an infection during a vaccine trial (Lanar *et al,* 1989) or in asymptomatic residents of endemic areas. However, sensitivities of 76-85% have been achieved in field studies in such areas (Holmberg *et al,* 1987, 1990). Enzyme-labeled synthetic DNA probes have been developed with the same detection limit as radio-labeled probes (approximately 100 parasites/μl), although this may necessitate longer incubation times (McLaughlin *et al,* 1987; Sethabutr *et al,* 1988). A probe which identifies *P. vivax* in blood samples has also recently been developed (Relf *et al,* 1990).

Probes which detect the species-specific regions of ribosomal RNA have been proposed as potentially sensitive tools for malaria diagnosis because of the large copy number of such molecules per cell (Waters and McCutchan, 1989; Lal *et al,* 1989). Such probes have yet to be tested appropriately on patients or residents in malarious areas.

Polymerase chain reaction (PCR) requires central laboratory facilities but is potentially more sensitive than DNA probes. Thus Tirasophon *et al* (1991) have developed a PCR primer combination that can detect a single *P. falciparum* parasite. This technique may be particularly useful for quality control of other assays, including blood smears, since the sensitivity of the latter is less than PCR.

Improvements in microscopy

The desire for improvement of microscopic diagnosis of malaria has led to the investigation of modifications or alternatives to Giemsa stain (Kawamoto *et al,* 1991; Makler *et al,* 1991; Jamjoom *et al,* 1991). Acridine orange is used to stain the Quantitative Buffy Coat (QBC) microhematocrit tubes, in which the density of infected erythrocytes leads to their concentration after centrifugation in a specific layer at the periphery of the tube which contains a central float (Levine *et al,* 1989). Parasites are detected by examination of this layer by fluorescence. This method was reported to be more sensitive than microscopy in a study of Kenyan malaria patients (Spielmann *et al,* 1988) and to have 96% sensitivity compared to microscopy in Thai hospital patients (Rickman *et al,* 1989). However, in field studies of asymptomatic individuals, the low parasitemias present the same problems for QBC tubes as they do for the DNA probes: the sensitivity was 77-79% compared to microscopy in such areas in Thailand (Rickman *et al,* 1989; Wongsrichanalai *et al,* 1991). Specificity exceeded 93% in these studies and when species determination was attempted it was possible in 73-83% of cases.

Immunoassays for antigen detection

ELISAs and radioimmunoassays for detecting malaria antigens were first developed in the 1970s (WHO, 1988). The initial tests, which were based on the inhibition of binding of relatively crude labeled antibody preparations to parasite extracts, suffered from the drawback of high false positive rates, presumed to be due to the persistence of antigen after resolution of an infection (Mackey *et al,* 1980; Avraham *et al,* 1983), but possibly also due to the crude nature of the antibodies used at that time. These difficulties have been partially overcome by the use of labeled monoclonal antibodies (MAbs) in inhibition assays; one assay which used *P. berghei* as antigen reported a sensitivity of 68.3% in detecting *P. falciparum* with 99.8% specificity (Avidor *et al,* 1987). Immunoradiometric sandwich assays with much higher sensitivity (Khusmith *et al,* 1988) unfortunately are unsuitable for field use because of the isotope requirement. One sandwich MAb-based antigen detection assay has been developed for the specific purpose of screening blood donors (Dubarry *et al,* 1990). Using two different MAb combinations, its sensitivity in detecting antigen in patients suffering their first attack of malaria was 72.2%, with 99.2% specificity. The test proved its usefulness in blood donor screening in combination with immunofluorescence (IFAT) for detection of antibody: antigen was detected in 5.3% of potential blood donors who had no anti-malaria antibody but who had recently travelled to malarious areas. A recently reported pan-species MAb-based ELISA gives sensitivities in the range of 87-98%, which approaches the results of routine microscopy

(Gao *et al,* 1991).

Immunoassays for antibody detection

Antibody detection by immunofluorescence assays is commonplace in some countries (*eg* China) as a means of confirming malaria diagnosis and/or for monitoring the effects of control programs or chemoprophylaxis. As a technique it is time-consuming, requires skilled operators and equipment, and has difficulties of standardization. Therefore a number of enzyme-based assays have been developed. A recent example is that of Sato *et al* (1990), an ELISA using the avidin-biotin amplification system for detection of antibodies to sonicated parasite extracts. The use of a precipitable substrate allows the test to be read (as an antibody titer) with the naked eye. Detailed field testing has not been carried out but initial studies indicated 100% sensitivity and 95% specificity with 25 prediagnosed Japanese malaria patients and 20 controls. Another assay not requiring an ELISA reader is the dot-immunobinding assay of Londner *et al* (1987) which uses sonicated multistage antigen of cultured *P. falciparum* on a nitrocellulose membrane. Modifications in antigen preparation by Wang *et al* (1989) resulted in 94.2% sensitivity using freeze-thawed parasite antigen extracts and 96.2% sensitivity using soluble antigen, in tests with 52 patients; specificity was 100% with 48 controls.

In this paper the usefulness of antibody detection ELISAs for the diagnosis of malaria in non-immunes is explored. Imported malaria is a rapidly increasing problem in Australia (Boreham and Relf, 1991). There are approximately 700 cases of imported malaria per year, of which about half are imported into Queensland. Roughly two-thirds are *P. vivax* and one-third *P. falciparum,* with the occasional *P. malariae* and *P. ovale.* The majority of these occur in non-immune Australian residents who travel to endemic countries for business or tourism. Tests which prove useful for imported malaria should also be applicable to non-endemic areas of malaria-endemic countries, although greater constraints in cost and technology may of course apply.

The antigens used in the present study include detergent parasite extracts of *P. falciparum* and *P. chabaudi; P. vivax* antigen is not readily available because it cannot be grown in culture. Because of the difficulty of standardization of whole parasite extracts, defined antigens such as synthetic peptides would be preferable and would also lend themselves to development of novel tests such as the 'Agen' test employing red cell agglutination (Rylatt *et al,* 1990). Initial screening of peptides from various antigens of *P. falciparum* indicated that 3 peptides from the N-terminal constant region of the merozoite surface antigen 2 (MAS2) (Saul *et al,* 1989) were potentially of value. These peptides (G3, G7, G23) were thus tested as antigens.

MATERIALS AND METHODS

Serum samples

Sera from malaria patients were obtained through the cooperation of doctors and pathology laboratories in Brisbrane. Details of the collection are given by Boreham and Relf (1991). The samples were obtained when blood slides were made for microscopic diagnosis. Samples were obtained from 51 cases of *P. falciparum,* 136 cases of *P. vivax,* 2 cases of *P. ovale* and 4 mixed infections of *P. falciparum* and *P. vivax.* Follow-up sera were obtained from 18 *P. falciparum* cases (2 serial samples in 4 cases), 36 *P. vivax* cases (more than one follow-up sample in 4 cases) and one *P. ovale* case. Follow-up sera were obtained between one day and 36 weeks after the initial sample, with the majority at 1-8 weeks. In analysis of the results, follow-up samples were only include if they were taken between 1 and 10 weeks after the first sample. Negative control sera from persons never exposed to malaria were obtained from the Red Cross Blood Bank, Brisbane.

Antigens

Parasite antigen was prepared from Percoll purified schizonts of *P. falciparum* FCQ27 strain, or from mouse blood infected with 20% parasitemia of *P. chabaudi* as follows. Erythrocytes were lysed with 0.16% saponin and the parasite pellet centrifuged and washed until all hemoglobin was removed. The pellet was resuspended in phosphate buffered saline (PBS) containing protease inhibitors to 5×10^8 parasites/ml, frozen and thawed 3 times, sonicated and centrifuged. The pellet was extracted with 0.5% NP40 in PBS containing protease inhibitors. The resulting supernatant was dialysed against PBS and stored at -70°C.

Peptide antigens with the following sequences were synthesized by the method of Houghten (1985):

G3: CKGTGQHGHB
G7: CMHGSRNNHB
G23: CLLNNSSNIB

Peptides were conjugated to MCS-BSA as described by Jones *et al* (1990) and used to coat ELISA plates at 5 μg/ml in PBS. Tests using the mixture of G3, G7 and G23 as antigen contained one-third of the standard concentration of each peptide.

ELISA method

Initial tests with immune serum pools and negative controls determined the optimum concentrations. Flat-bottomed PVC plates (Flow Labs, activated) were coated at 4°C overnight (or for 2 hours at 37°C) with 50 μl per well of antigen diluted in PBS. Plates were washed twice with PBS and then blocked with 5% low-fat milk powder in PBS at 37°C for 2 hours. After washing twice with PBS, 50 μl of serum at 1 : 100 dilution in PBS containing 0.05% Tween 20, 5% low-fat milk powder and 0.1% BSA were added to each well. A control no-antigen well was included for each serum, as well as quadruplicate controls of positive and negative pooled sera (10 sera/pool) and a no-serum control. Plates were incubated for 2 hours at 37°C and then washed 4 times with PBS/0.05% Tween. Horseradish peroxidase-linked anti-human antibody diluted in PBS with 0.05% Tween and 5% low-fat milk powder was added at 50 μl per well. Three types of anti-human antibody conjugates were tested. Anti-human whole Ig (Kierkegard and Perry Labs) was used at 1 : 1000 dilution, while anti-human IgG and IgM Sigma were used at 1 : 500 dilution. Plates were incubated at 37°C for 1 hour and washed 5 times with PBS/0.05% Tween before 50 μl per well of ABTS substrate were added (Kierkegard and Perry Labs, two-component system). Optical densities (ODs) were read at 405 nm after 30-90 minutes at room temperature.

Data analysis

Correction for background binding of sera to the ELISA plate was achieved by subtracting the 'no-antigen' OD for each serum from the OD in the presence of each antigen. To achieve standardization between plates, one plate for each antigen series was selected as standard. OD values on each other plate in the series were multiplied by the ratio between the positive controls (mean of 4 wells) on that plate and the standard plate. For each antigen the mean and standard deviation of the ODs given by Brisbane control sera (n = 125-143) were determined. Sera were classed as positive for a particular antigen if their ELISA value exceeded 2 standard deviations of the mean of the Brisbane control sera. Sensitivity values were calculated as the percentage of sera from malaria cases which were positive with an antigen or group of antigens. Specificity values were determined as the percentage of control (non-exposed) sera which were negative with each antigen or group of antigens. Differences in the frequencies of positive and negative sera detected by each antigen or antigen group were compared by chi-square tests.

RESULTS

Initial screening for diagnostic potential was performed with 13 peptides from a range of malaria antigens (FIRA, MESA, HRP2, GBP, RESA, *P. vivax* CS protein, *P. malariae* CS protein) and 76 peptides from the *P. falciparum* MSA2 antigen. Three from the MSA2 antigen (G3, G7, G23) were selected for further testing and comparison with parasite extracts.

Six antigens were compared for their ability to detect antibody at the time at which the patient presented for diagnosis of malaria, and in the follow-up serum samples. These antigens were *P. chabaudi* extract (Pc), *P. falciparum* extract (Pf), peptide G3, peptide G7, peptide G23 and a mixture of these 3 peptides (pepmix). Four further percentage sensitivities were calculated according to the following criteria:

(1) Positive on either of the parasite extracts ('Pc or Pf')
(2) Positive on either of the 3 peptides ('G3 or G7 or G23')
(3) Positive on peptides G3 or G7 ('G3 or G7')
(4) Positive on either Pc or peptide G3 or peptide G7 ('Pc or G3 or G7').

In Table 1 are shown the resulting sensitivities to the 10 antigens or antigen combinations for the *P. falciparum* and *P. vivax* cases.

Table 1

Sensitivity of the ELISA with anti-total human Ig for acute and follow-up sera from malaria cases in Brisbane.

Antigen	Percent positive			
	P. falciparum (%)		*P. vivax* (%)	
	Acute	Follow-up	Acute	Follow-up
	N=48	N=6	N=132	N=36
Pc	66.7	83.3	79.4	80.6
Pf	68.1	100.0	43.2	47.2
Pc or Pf	76.6	100.0	83.2	83.3
G3	56.3	83.3	53.8	62.9
G7	54.2	66.7	49.2	51.4
G23	43.8	66.7	50.8	54.3
Pepmix	62.5	100.0	62.9	68.6
G3 or G7 or G23	68.8	100.0	71.2	85.7
G3 or G7	64.6	83.3	62.9	81.5
Pc or G3 or G7	77.1	100.0	88.6	91.7

Results with the total sample of sera from acute cases will be considered first. Parasite antigens were more effective than peptides in detecting acute cases of *P. falciparum* and *P. vivax* (Table 1). There was no difference between the sensitivity of Pf and Pc extracts in detecting *P. falciparum* acute cases ($\chi^2=0.01$, $p=0.9431$), but Pc antigen was markedly superior for *P. vivax* acute cases ($\chi^2=34.8$, $p<0.0001$). Thus the Pc antigen is suitable for detection of antibodies to both species. Some increase in sensitivity over the Pc antigen was obtained by the use of the combined parasite antigen positivity rate, 'Pc or Pf', but the differences were not statistically significant in either *P. falciparum* or *P. vivax* cases.

Amongst peptide antigens, peptide G3 gave the highest positivity rate for both *P. falciparum* and *P. vivax* cases (Table 1). The combined peptide positivity rate ('G3 or G7 or G23') was higher, but only significantly so for *P. vivax* cases ($\chi^2=7.82$, $p=0.0052$). Results with the pepmix antigen, in which the 3 peptides were mixed in the same plate were not quite as good as the combined results from the 3 separate tests; removing G23 from the combined results ('G3 or G7' only) caused a non-significant drop in the positivity rates ($\chi^2=0.05$, $p=0.8286$ for *P. falciparum;* $\chi^2=1.71$, $p=0.1904$ for *P. vivax.* However, using the combined parasite/peptide data ('Pc or G3 or G7') increased the sensitivity significantly over the 3 peptide combination for *P. vivax* ($\chi^2=4.22$, $p=0.0399$) although not for *P. falciparum* ($\chi^2=0.47$, $p=0.4908$). The rates for 'Pc or G3 or G7' were not significantly higher than those for 'Pc or Pf' for either malaria species, nor than Pc alone for *P. falciparum.* However, for *P. vivax,* sensitivity was 88.6% using 'Pc or G3 or G7' compared to 79.4% for Pc alone, which approached statistical significance ($\chi^2=3.53$, $p=0.0603$).

Follow-up cases of *P. falciparum* were too few for statistical analysis, but sensitivity was 100% with 'Pc or Pf', 'Pc or G3 or G7', 'G3 or G7 or G23' and pepmix combinations of antigens (Table 1). In *P. vivax* cases the results were similar to those observed in acute sera, with higher positivity rates for all antigens.

Over half of the persons presenting with suspected malaria had had a previous attack of the disease. Therefore the sensitivity of the assays was assessed in those for whom information about previous history of malaria was available (Table 2). In *P. falciparum* all antigens were at least effective in detecting antibody in those for whom this was their first attack. The best sensitivity was achieved by the combined peptide results ('G3 or G7 or G23') or the combined parasite/peptide

Table 2

Sensitivity of the ELISA using anti-total human Ig conjugate with sera from acute malaria cases.

Antigen	Percent positive			
	P. falciparum (%) Previous malaria		*P. vivax* (%) Previous malaria	
	No	Yes	No	Yes
	N = 11	N = 14	N = 24	N = 58
Pc	36.4	85.7	62.5	86.0
Pf	36.4	92.3	20.8	58.6
Pc or Pf	36.4	100.0	66.7	89.5
G3	45.5	57.1	79.2	41.4
G7	36.4	57.1	62.5	43.1
G23	34.4	50.0	62.5	36.2
Pepmix	36.4	92.9	79.2	55.2
G3 or G7 or G23	54.6	85.7	87.5	63.8
G3 or G7	45.5	78.6	83.3	55.2
Pc or G3 or G7	54.6	92.9	87.5	91.4

results ('Pc or G3 or G7'), both of which had a sensitivity of 54.6%, but the small sample size meant that no differences between any of the antigens were statistically significant. For *P. vivax,* on the other hand, the peptide antigens, especially G3, were more sensitive in detecting those cases without previous malaria history than those who had previously been infected (79.2% versus 41.4% for G3, $\chi^2 = 8.26$, $p = 0.0040$). Both the combined peptide and combined parasite/peptide antigens gave a high sensitivity (87.5%) in *P. vivax* first-attack cases. The advantage of using the combined parasite/peptide antigen ('Pc or G3 or G7') is demonstrated by the *P. vivax* cases with previous malaria history, in which this combination achieved 91.4% sensitivity compared to 63.8% for the peptide-only combination ($\chi^2 = 11.15$, $p = 0.0008$).

Sera from the 4 mixed *P. falciparum/P. vivax* and 2 *P. ovale* infections were examined separately for their reactions to the antigens Pc, G3, G7 and G23. Three out of the 4 mixed infections were positive with all 5 antigens and the other was positive with the 3 peptide but negative on the parasite extract. Both *P. ovale* cases were positive on the Pc antigen and one was also positive on G3 and G23, as was follow-up serum on the same case. Thus all of these infections were detected by the criteria 'G3 or G7', 'G3 or G7 or G23', or 'Pc or G3 or G7'.

Selected antigens (Pc, Pf, G3 and G7) were tested with the serum panel using anti IgG and IgM specific conjugates. As reagents in an antibody detection test aiming at high sensitivity, these conjugates performed badly compared to the anti-total Ig conjugate, as might be expected (Tables 3, 4). For example, the highest sensitivity achieved for *P. falciparum* acute cases was 54.2% with the Pf antigen (Table 3), compared with 68.1% using the anti-total Ig conjugate Table 1). However, use of the anti IgG and IgM conjugates gave rise to some interesting observations, especially with the peptide antigens, when the results were examined in terms of previous malaria history. With the parasite extracts (Pc, Pf) results were as expected, namely higher percentages of sera from persons with previous exposure were positive for IgG than those with a primary attack. This positivity rate was as high as 92.9% in *P. falciparum* cases using Pf antigen (Table 3). On the whole, the converse was true for IgM, with higher positivity rates to parasite antigens in primary attack sera, although an exception was seen in *P. vivax* cases using Pf antigen, where positivity rates were equal in those with and without previous malaria history (Table 4).

Table 3

Sensitivity of the ELISA using anti-human IgG and IgM conjugates with acute sera from *P. falciparum* cases.

Antigen	Percent positive					
	IgG (%) Previous malaria			IgM (%) Previous malaria		
	No	Yes	Total	No	Yes	Total
	N=11	N=14	N=48	N=11	N=14	N=48
Pc	9.1	42.9	29.2	45.5	35.7	39.6
Pf	18.2	92.9	54.2	45.5	28.6	35.4
G3	9.1	42.9	25.0	36.4	21.4	27.1
G7	27.3	7.1	18.8	45.5	64.3	54.2

Table 4

Sensitivity of the ELISA using anti-human IgG and IgM conjugated with sera from acute cases of *P. vivax*.

Antigen	Percent positive					
	IgG (%) Previous malaria			IgM (%) Previous malaria		
	No	Yes	Total	No	Yes	Total
	N=27	N=58	N=135	N=27	N=58	N=135
Pc	37.0	51.7	44.4	48.2	32.8	34.8
Pf	22.2	58.6	39.3	25.9	25.9	23.0
G3	22.2	12.1	11.1	48.2	20.7	31.1
G7	40.7	43.1	37.8	37.0	29.3	34.8

Peptide G3 antigen also gave the expected pattern of isotype-specific response with *P. falciparum* cases (Table 3). With peptide G7, on the other hand, response rates in first attack cases were higher for IgG than for IgM than in secondary attacks. In *P. vivax* a more confusing picture is presented (Table 4). Both peptides G3 and G7 gave the expected response of higher IgM rates in primary cases, but peptide G3 also gave higher IgG rates in primary cases whilst peptide G7 had IgG rates which were similar in both primary and secondary cases. Because of the restricted sample of sera for which previous history is known, none of these differences achieved statistical significance. Nevertheless, this trend towards relatively high IgG responses to peptides in first-attack cases of *P. vivax* is presumably responsible for the high efficiency of these peptides in detecting such cases mentioned above (Table 2).

Specificity of the ELISA with sera from Brisbane residents who had never been exposed to malaria was greater than 94% for all single antigens tested (Pc, Pf, G3, G7, G23) and with pepmix, using either the anti-total Ig or anti IgG or IgM conjugates (Table 5). Increasing the sensitivity of the test by combining antigens was achieved at the expense of some specificity (90.4% for combined peptides, 91.1% for 'Pc or G3 or G7').

Table 5

Specificity of the ELISA using sera from persons never exposed to malaria.

Antigen	Percent negative	Number
Pc	98.6	142
Pc (IgG)	98.4	125
Pc (IgM)	94.4	125
Pf	95.7	139
Pf (IgG)	98.4	125
Pf (IgM)	96.0	125
Pc or Pf	94.2	139
G3	97.0	135
G3 (IgG)	98.4	125
G3 (IgM)	95.2	125
G7	94.1	135
G7 (IgG)	99.2	125
G7 (IgM)	96.8	125
G23	97.0	135
Pepmix	95.6	135
G3 or G7 or G23	90.4	135
G3 or G7	92.6	135
Pc or G3 or G7	91.1	135

DISCUSSION

Tests with detergent extracts of *P. chabaudi* and *P. falciparum* showed there to be little advantage in using *P. falciparum* antigen for acute cases of malaria, since *P. chabaudi* antigen gave equivalent sensitivity with *P. falciparum* cases while being markedly superior for *P. vivax* cases. Combinations of peptide antigens G3, G7 and G23 were surprisingly sensitive, especially for first-attack cases of *P. vivax* where they achieved a sensitivity of 87.5%. Combination of the results of the 3 assays (Pc, G3, G7) gave the highest positivity rates for both acute and follow-up sera. This combination detected 77.1% of *P. falciparum* acute cases and 88.6% of *P. vivax* acute cases.

Most of the serum samples in this study were obtained from patients with low parasitemia (Boreham and Relf, 1991), especially for the group who were not previously infected. For this group, therefore, this is a stringent test of the ELISA. Nevertheless, whilst the ELISA described here matches the sensitivity of previously described tests (see Introduction), blood slide examination remains the best readily available way of diagnosing malaria in this group. In follow-up samples, the ELISA performed much better, albeit with a small sample, detecting 100% *P. falciparum* and 91.7% of *P. vivax* cases. The high positivity rate of the test in follow-up samples from patients who were rapidly treated with a curative regime following a low initial parasitemia suggests its use as a tool for identifying persons with recent history of malaria, for epidemiologic purposes, identification of asymptomatic carriers or retrospective diagnosis.

False positive rates for the assays varied according to the antigen, but were less than 10% even for rates obtained using combinations of antigens. In this potentially life-threatening disease where it is considered sensible to err on the side of caution, this can be regarded as acceptable; decreasing the false-positive rate by using a more stringent cut-off value would be achieved as the expense of some sensitivity. There was no advantage in sensitivity from using anti IgG or IgM specific conjugates in the ELISAs, although this study revealed unexpected patterns in the isotype-specific response to peptides which deserve further study.

In conclusion, this study has demonstrated the usefulness of a combination of 3 antigens in malaria antibody detection ELISAs: *P. chabaudi* parasite extract and peptides G3 and G7 from the *P. falciparum* MSA2 antigen. Whilst it would be desirable to have a single antigen for the assay, at present this combination of antigens represents a good compromise to ensure the detection of malaria in both primary and secondary attack cases of *P. falciparum* and *P. vivax*.

Antigen detection assays would be expected to be the most useful field tests for detecting a current malaria infection, yet species-specific MAb-based ELISAs for antigen detection of have mostly reported sensitivities considerably less than microscopy (Dubarry *et al*, 1990; Tharavanij, 1990) possibly due to the problem of antigenic variability of the parasite. However, a recent study with an ELISA using a pan-species MAb reports positivity rates of 87-98% (Gao *et al*, 1991), indicating that antigen detection by appropriate ELISA should present a potential field diagnostic option, especial

ly if developed in a rapid test format. The potential of DNA and RNA probes has been recognized (Barker *et al,* 1990; Waters and McCutchan, 1989) as has that of PCR (Tirasophon *et al,* 1991), but facilities for performing these tests are not yet available at appropriate locations in most endemic countries. Even with development of better parasite/antigen detection methods, antibody detection ELISAs, especially if translated to a rapid dot-ELISA format, have a place in malaria control programs as a substitute for IFAT for monitoring transmission and identifying remaining reservoirs of infection.

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