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Evaluation of a Malaria Antibody ELISA and Its Value in Reducing Potential Wastage of Red Cell Donations from Blood Donors Exposed to Malaria, with a Note on a Case of Transfusion-Transmitted Malaria

Abstract

Background and objectives: Blood donations are often wasted for lack of a satisfactory procedure to evaluate donors potentially exposed to malaria. **Materials and methods:** We evaluated a commercial ELISA for the detection of antibodies to malaria and compared it with an immunofluorescent antibody test (IFAT). **Results:** When 5,311 sera from routine non-exposed donors were tested, 24 (0.45%) were positive by the ELISA, using a Plasmodium falciparum antigen. Seventeen were subjected to confirmatory testing but none were positive by IFAT. Of 1,000 donors potentially exposed in endemic areas 15 (1.5%) were repeatably reactive by ELISA. 10 of these were tested by IFAT and 2 were positive. When 150 patients attending the Hospital for Tropical Diseases in London with acute malaria were tested, 73% of those infected with P. falciparum were repeatably reactive for malarial antibodies by ELISA and 56% with Plasmodium vivax. Of 88 stored clinical sera tested by both IFAT and ELISA 56 were positive by IFAT and of these 52 (93%) were positive by ELISA. **Conclusion:** The ELISA is sufficiently sensitive and specific to screen at-risk donors. Its use could safely retrieve 40,000 red cell units currently discarded each year in Great Britain.

Introduction

The first case of transmission of malaria by blood transfusion was reported in 1911. Bruce-Chwatt [1, 2] reviewed worldwide data recorded from 1911 to 1979 during which the reported incidence increased from about 6 to 145 cases per year. In the early years *Plasmodium vivax* was the commonest species involved but in the 1950s *Plasmodium malariae* predominated followed by *P. vivax, Plasmodium falciparum,* mixed infections and *Plasmodium ovale.* More recent data from the 1970s showed *P. vivax* as the commonest species followed by *P. malariae* and *P. falciparum,* the proportion of the last having risen substantially.

Although transfusion malaria is rare in the UK the pattern of infection introduced by people travelling from endemic areas has shown similar changes. Between 1984 and 1986 there were more cases of *P. vivax* than *P. falciparum,* since then there have been more cases of falciparum malaria [3]. The proportion of malaria due to *P. falciparum* has risen from 37% in 1984 to 55% in 1993, reflecting the fact that in

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1993 over twice as many infections were acquired in Africa as in Asia. The last three cases of transfusion malaria reported by the North London Blood Transfusion Centre were all due to *P. falciparum*. In the absence of a practical screening test, transfusion centres reduce the risk of transmission by discarding red cell donations from potentially infected donors according to their medical and travel history. The guidelines used are complicated and may result in a 3-year period during which red cell donations are discarded. Since most of the donors involved have not in reality been infected there is significant unnecessary wastage. The North London Blood Transfusion Centre alone discards the non-plasma components from 6,000 such units each year. In the UK there is an annual loss involving about 40,000 units [in-house National Blood Authority data].

A reliable and efficient screening test for malaria could reduce unnecessary rejection of donations while minimising the risk of transmission. Examination of blood films is incompatible with transfusion microbiological practices and, in any case, is not sensitive enough to exclude infection. Although an ELISA for malarial antigen detection has been devised, it detects *P. falciparum* only and does not improve on the sensitivity of blood film examination [4]. Malaria antibody screening by the immunofluorescent antibody test (IFAT) or ELISA, both using *P. falciparum* antigen, has been suggested [5] as some cross-reactivity with antibodies from individuals infected with the other species occurs. Although the IFAT is considered the standard diagnostic antibody test [6], a simpler microplate-based ELISA would be preferable for mass screening of donations. Test processing, reading and information transfer can be automated and an ELISA would be compatible with other transfusion screening procedures.

We have evaluated a commercial ELISA for malaria antibodies and compared it with a reference ELISA [7] performed in-house at the Nuffield Laboratories for Comparative Medicine, London and an IFAT [8]. We also assessed the value of the commercial ELISA in simplifying the eligibility protocol for potentially infected donors to thereby reduce the number of rejected red cell donations.

Methods

The Malaria Antibody ELISA

The indirect malaria antibody ELISA method was used and kits were obtained from Launch Diagnostics. (The kits are currently produced by Cellabs pty, 27 Dale Street, Brookvale, NSW 2100 Australia.) The assay is an antiglobulin ELISA using microplates coated with *P. falciparum* antigen. Antigen is prepared by sonication of washed *P. falciparum*-parasitised erythrocytes and centrifugation at 10,000 *g* for 30 min. The supernatant is stored at –70 °C until use [7, 9]. 10 µl of serum or plasma samples are added to 100 µl of PBS-Tween in the coated wells and incubated for 1 h at 37 °C to allow antibody to bind to the antigen. The plate is then washed 3 times with PBS-Tween. 100 µl of conjugate (antihuman IgG labelled with horseradish peroxidase) are added and the plate incubated for 1 h at 37 °C. The conjugate binds to any antibody present. The wells are washed 3 times in PBS-Tween and *o*-phenylenediamine plus hydrogen peroxide added for 15 min at room temperature. The production of orange colouration indicates the presence of malaria antibody. 50 µl of hydrochloric acid are added to stop the reaction and the absorbance is read at 492 nm on an ELISA reader.

Panels of Sera Tested

To evaluate the reproducibility, sensitivity and specificity of the assay the following samples were tested.

Reference Samples. To test precision and reproducibility two panels of reference sera were tested. Panel 1 comprised 2 kit reference controls and 6 sera with confirmed antibodies to *P. falciparum*. These were tested on six occasions at a reference laboratory. Panel 2 comprised 2 kit reference controls and 1 mid-range positive serum. These were tested by the four participating British Blood Transfusion Centres (A, B, C, D) and the reference laboratory.

Clinical Samples. (1) 873 blood samples taken at presentation from patients who had attended the Hospital for Tropical Diseases (HTD) in London were tested by ELISA. Of these, 150 had acute malaria diagnosed by positive blood films, while 723 had negative blood films but had a differential diagnosis which included malaria as a possibility. (2) 88 patients' sera, which had been sent to HTD for malaria antibody testing as part of their clinical care and subsequently stored, were tested both by IFAT and ELISA. IFAT testing was performed quantitatively to obtain a titre [8, 9].

Tropical Area Blood Donors

The four participating transfusion centres tested a total of 1,000 sera from blood donors who had been potentially exposed to malaria in tropical areas and excluded from red cell donation. Initially and repeatably reactive samples from three centres were tested by an in-house ELISA at the reference laboratory and by IFAT at HTD.

Routine Blood Donors

5,311 sera from routine blood donors taken at the four transfusion centres were tested. These donors had either never been exposed to malaria or, following previous exposure, had been reinstated as full donors. Initially reactive samples were retested in duplicate. Initially reactive and repeatably reactive samples from three centres were tested at the reference laboratory by reference ELISA [7] and at HTD by IFAT. In addition, 75 sera from routine donors found to be negative by Launch ELISA were sent for confirmatory testing by reference ELISA and IFAT.

Results

Reference Samples

Testing of panel 1 on six occasions by the reference laboratory showed good precision with little variation in results from run to run (table 1). One sample with low antibody

Table 1. Results of the reference laboratory testing serum panel 1 by Launch ELISA on six occasions

	Launch ELISA optical density result					
	1	2	3	4	5	6
1	0.49	0.52	0.49	0.61	0.62	0.63
2	0.60	0.61	0.71	0.73	0.71	0.70
3	0.32	0.36	0.45	0.43	0.37	0.34
4	0.67	0.72	0.86	0.86	0.84	0.87
5	0.67	0.68	0.80	0.81	0.78	0.71
6	0.64	0.64	0.78	0.74	0.69	0.72
Kit negative control	0.04	0.03	0.05	0.05	0.04	0.04
Kit positive control	0.96	0.98	1.10	1.12	1.10	1.11

Table 2. Mean optical density results after participating laboratories tested serum panel 2 by Launch ELISA in triplicate

Centre	Kit reference control samples		Mid-range reactive sample
	strongly reactive	negative	
A	1.20	0.097	0.53
B	1.14	0.099	0.47
C	1.17	0.064	0.43
D	1.16	0.066	0.42
Reference laboratory	1.20	0.060	0.43

Table 3. Launch ELISA results on blood from patients attending HTD, London

Blood film results	Samples tested	Repeatably reactive by Launch ELISA
P. falciparum	114	83 (73)
P. vivax	25	14 (56)
P. malariae	1	0
P. ovale	10	6 (60)
Negative	723	142 (20)

Figures in parentheses represent percentage.

Table 4. Comparison of Launch ELISA with *P. falciparum* IFAT

IFAT titre	Number of sera IFAT-positive	Number of sera Launch ELISA-positive
1/20	7	6
1/40	9	9
1/80	14	13
1/160	23	23
Total	53	51

levels was consistently reactive. When the four transfusion centres tested panel 2, highly reproducible results were obtained with laboratories reporting optical densities virtually identical to those of the reference laboratory (table 2).

Clinical Samples

(1) Using ELISA to test sera from the 150 patients at the HTD with confirmed malaria, a repeatably reactive result was obtained in 73% with *P. falciparum* infection and in 56% infected with *P. vivax*. *P. ovale* infections were also detected but the numbers tested were small (table 3). Results for the 723 acute blood samples from patients in whom malaria was part of the differential diagnosis but with a negative blood film showed that 20% were reactive for malaria antibodies by commercial ELISA.

(2) Testing the 88 stored sera, 56 were positive by IFAT and 52 of these (93%) were detected by the ELISA. Of the 56 IFAT-positives, 53 had titres against *P. falciparum* and 51 (96%) were positive by ELISA (table 4). 32 of the 88 sera were negative by IFAT. 31 of the 32 were negative by ELISA.

The two positive *P. falciparum* IFAT results missed by ELISA were probably falsely negative. One of these was from a patient with a *P. falciparum* IFAT positive at a titre of 1 in 20 (i.e. weak positive) who had returned 3 weeks previously from an 8-month visit to Africa where he had suffered from malaria, species unstated. The other patient had a *P. falciparum* titre positive at 1 in 80 and presented at HTD with falciparum malaria (<0.001% parasitaemia) 2 days after returning from the Gambia. A blood sample taken 18 days after the start of his illness was positive in the IFAT at 1 in 80 but negative by commercial ELISA. Had either of these patients with probably falsely negative ELISA results presented as blood donors, both would have been excluded on the basis of potential exposure to malaria within the previous 6 months.

Serum from 1 patient was positive by IFAT both to *P. vivax* and *P. malariae* but negative by commercial ELISA. This patient had suffered vivax malaria and 10 months later presented with a mixed *P. falciparum* and *P. vivax* infection. A serum sample taken 4 days after presentation was IFAT-positive at 1 in 320 to *P. vivax* and at 1 in 40 to *P. malariae* but negative by IFAT to *P. falciparum*. It was also negative in the commercial ELISA. Although the patient had not been to the tropics for 10 months, he was unwell and febrile at pre-

Table 5. Results of testing tropical area blood donors by Launch ELISA, reference ELISA and IFAT

Transfusion centre	Samples tested by kit ELISA	Kit ELISA samples		Kit reactives positive	
		reactive in 1 test well	reactive in both test wells	by reference ELISA	by IFAT
A	250	6	5	ND	ND
B	250	3	3	2	2
C	250	7	7	4	0
D	250	0	0	0	0
Total	1,000	16 (1.6)	15 (1.5)	6	2

Figures in parentheses represent percentage. ND = Not determined.

Table 6. Results of testing routine blood donors by Launch ELISA, reference ELISA and IFAT

Transfusion centre	Samples tested by Launch ELISA	Initially reactive by Launch ELISA	Repeatably reactive by Launch ELISA	Kit reactives positive	
				by reference ELISA	by IFAT
A	1,250	10	7	ND	ND
B	1,250	9	6	4	0
C	1,555	6	6	4	0
D	1,256	6	5	3	0
Total	5,311	31 (0.58)	24 (0.45)		

Figures in parentheses represent percentage. ND = Not determined.

sentation and thus would have been excluded from blood donation. 1 patient with antibodies at 1 in 40 to *P. malariae* in the IFAT was negative by ELISA. A blood sample was negative by polymerase chain reaction for *P. malariae* [Warhurst, pers. commun.].

Tropical Area Blood Donors

Screening of the 1,000 tropical area donors by kit ELISA revealed 15 repeatably reactive samples (1.5%). Confirmatory testing on 10 of these showed 6 were positive by reference ELISA and 2 positive by IFAT (table 5).

Routine Blood Donors

Of the 5,311 donors tested by commercial ELISA, 31 were initially reactive (0.58%) and 24 repeatably reactive (0.45%, table 6). Following confirmatory testing of 17 repeatably reactive samples from 3 centres, 11 were considered positive by the reference ELISA, but none were positive by IFAT (table 6). Confirmatory testing of 75 sera from routine donors negative by commercial ELISA showed that all were negative by reference ELISA and IFAT.

Discussion

The results of testing reference samples showed the commercial ELISA to have good inter- and intralaboratory consistency and reproducibility. The ELISA detected 93% of those clinical samples found positive by IFAT. With respect to these aspects, the kit is potentially suitable as a screening assay for selected at-risk donors at transfusion service testing centres in non-malaria endemic areas.

With clinical samples from the HTD, the ELISA detected 73% of acute clinical *P. falciparum* infections and 56% of those currently infected with *P. vivax* with clinical symptoms. Even though the test uses falciparum antigen there was useful cross-sensitivity in detecting vivax antibodies. It must be borne in mind that most of the samples were from patients suffering from *acute* malaria and antibodies may not be detectable during the first few days of symptoms [10]. This suggests a very high level of sensitivity for the assay. Similarly, Draper and Sirr [10] found that 78% of UK residents suffering from their first attack of malaria had antibodies detectable by IFAT to *P. falciparum* antigen within 1 week after malaria had been diagnosed by blood film. The proportion of individuals positive for malaria antibodies increased from 1 to 4 weeks after onset and then decreased. It

must be emphasised that the ELISA test is not intended for the clinical diagnosis of acute malaria; blood film examination remains the method of choice. We would have preferred to test samples from convalescent patients who would have been a group more comparable to at-risk donors, of whom more would be expected to have antibodies, but we could not obtain such samples since patients are not followed up long term. We are confident the test is sufficiently sensitive in detecting infected donors if sufficient time is allowed for the development of antibodies following exposure. A donor with acute infection but a negative test in the 'window' period would almost certainly be too ill to consider donating blood. A semi-immune donor who might be asymptomatic, but parasitaemic, would be expected to have detectable antimalarial antibodies [10].

It is noteworthy that 20% of the patients with negative blood films, not suffering from acute malaria, were reactive by ELISA. This reflects the high prevalence of past infection in the patient population at the HTD. Many of these patients had lived in or had visited malaria endemic areas.

In screening tropical area donors by kit ELISA we found 1.5% to be repeatably reactive. Of the 10 samples positive by commercial ELISA 2 were positive by IFAT. The status of the discrepant results is uncertain. Whether they were true- or false-positive, the results show that 98.5% of the donors exposed to the risk of malaria had a negative ELISA test. With no evidence of infection, their non-plasma components were unnecessarily discarded under the eligibility protocol in use at the time.

Of the routine donors tested by the commercial ELISA, 24 were repeatably reactive. These donors had not been recently exposed to malaria so most of these reactives may have been falsely positive. Of 17 reactives tested by IFAT none were positive. Some donors may have had antibody from old infections and been reinstated as red cell donors following periods of exclusion. We believe the observed repeatably reactive rate of 0.45%, although high, is acceptable for a screening test for a minor proportion of the donor population selected for possible malaria exposure, since a reactive result in this instance does not carry the kind of critical clinical significance for the donor as is the case with HIV or hepatitis B infections.

If an antibody ELISA is considered for screening purposes the timing of the test must be carefully chosen. Sufficient time following exposure must elapse to allow for the variable incubation period of each malarial species and for the development of antibody. We suggest screening 6 months after the last exposure, both for returned travellers and for citizens of endemic areas arriving in the UK. Cases of malaria do occur more than 6 months after last exposure and are usually due to *P. vivax*. In 1992 and 1993, of 829 cases of vivax malaria for which information was available, 286 presented more than 6 months after arrival in the UK [3]. Only 7 of 1,434 cases of falciparum malaria occurred after 6 months. However, those without significant malarial immunity are likely to be symptomatic and thus either unlikely to attend a donor clinic or, should they attend, be rejected when questioned about their health. The few late cases that are asymptomatic, likely as the result of previous immunity from pre-existing antibody, would give a positive reaction in the assay should they donate.

It should be noted that with *P. vivax*, delayed attacks of malaria may occur 8 or 9 months after the last exposure and, with some strains of *P. vivax*, primary attacks can occur after 18 months [11]. This is due to activation of hypnozoites hitherto dormant in the liver. Neither hypnozoites nor pre-erythrocytic schizonts give rise directly to clinical illness, and neither would be expected to produce positive antimalarial ELISA or IFAT tests. Thus a donor would be asymptomatic until parasites had begun multiplication in the erythtrocytes in the peripheral blood. Even a 1-year exclusion period would not eliminate this possibility.

Testing at 6 months would shorten donor exclusion periods and make additional red cell units available. Other workers have argued for a shortening of deferral periods. In the USA Nahlen et al. [12] studied information on 22 of 32 donors implicated in cases of transfusion-transmitted malaria. They argued that shortening the deferral period from 3 years to 6 months for US travellers would still have prevented all but 1 of the 22 cases, equivalent to a risk of 1 additional case every 33 years. Admittedly, the risk of transfusion transmission of malaria is greater in the UK. Compared with the USA, the UK has almost twice as many cases of imported malaria in a population of 58 million. In 1991, in the United States and its territories with a population of approximately 255 million, 1,046 cases of malaria were recorded: 43% were due to *P. vivax*, 39% to *P. falciparum*, 6% *P. malariae* and 2% *P. ovale*, with 9% unspecified [13].

A combination of shortened deferral period and addition of serological testing would provide added security compared with history taking alone. In March 1994, a case of transfusion-transmitted falciparum malaria occurred when a female African donor living in London donated 2 months after a visit to Ghana. Her serum was later found to contain antimalarial antibodies by IFAT and by ELISA. A history of foreign travel was not elicited. If it had been obtained, a 6-month exclusion period would have excluded the donor from donation. When history taking is difficult or the degree of risk following travel uncertain, antibody testing would provide an additional safeguard. In the two previous

Table 7. Proposed action on the result of a positive malaria ELISA screening test

Test results	Action
ELISA screen-reactive or borderline	Repeat ELISA + IFAT
ELISA positive IFAT negative	Exclude from further donation No medical follow-up
ELISA positive IFAT positive	Clarify history; last possible exposure to malaria (i.e. last visit to malarial area)?
>2 years	Exclude from further donation No medical follow-up
6 months to 2 years	Review in local infectious diseases unit or Advise donor: positive antibody test; will need investigation if febrile in the next year or Inform GP of antibody result and suggest options as above

Long-term follow-up: for all positive ELISA results, whether or not confirmed by IFAT: option to re-attend at 3 years and retest (significant number will have lost antibody if no further exposure has occurred). If antibody negative at retest, re-instate to donor panel if not excluded by current criteria.

cases of transfusion-transmitted falciparum malaria reported by NLBTC, one was due to the lack of a full travel history but would have been prevented by a 6-month exclusion period, while the other was due to clerical error [14]. Had an inclusive policy for antibody testing been adopted, we feel these cases would have been most unlikely to be missed.

From our results, 1.5% of at-risk donors would be reactive, equivalent to about 1,200 donors in Great Britain each year. The most cost-effective way to manage these donors requires formal definition and agreed protocols. Many of them would be falsely positive and confirmatory testing by reference ELISA or IFAT may accurately distinguish true positives. These true positives might be permanently excluded from all future red cell donations or retested after a further exclusion period in an attempt to reinstate them following a negative result. The cost of screening and referral tests would be offset by the greater availability of red cell units.

If eligibility of at-risk donors were based on screening by ELISA at 6 months, we calculate that about 40,000 red cell donations, currently discarded each year in Great Britain, would become available for safe transfusion. Furthermore, the inherent complexity of donor exclusion following malarial exposure could be greatly simplified with a consequent reduction in the opportunities for error. It should be stressed that neither the current exclusion criteria nor serological testing can give an absolute guarantee against malarial transmission by transfusion. However, a system incorporating the selection of donors with *any* possible risk of malaria exposure with subsequent testing by antimalarial antibody ELISA would retrieve a substantial number of units for full component processing, without detracting from the current safety of the blood supply. A suggested course of action is outlined in table 7.

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