Malaria - a focus on screening

by Colin Knox & Nigel Appleton

Because only one malaria parasite in donated blood can lead to disease in the recipient, in many non-endemic countries there is currently a deferral policy for potential blood donors who have recently either visited or resided in a malaria endemic area. The rise in the number of donors who fall into this risk category is threatening the sufficiency of blood stocks, which has led to the development of a new enzyme immunoassay with a high degree of sensitivity and specificity to both Plasmodium falciparum and Plasmodium vivax. Trials with the new EIA have been successful, and its use should minimise the unnecessary deferral of potential blood donors.

The relative ease and speed of modern travel and migration means that "imported" cases of infectious diseases may present in any country. This is especially important in the case of malaria due to its high and growing endemicity in many areas of the world and of particular relevance to the safety of blood transfusions.

The risk of transmitting transfusion-associated malaria is complicated by the long and relatively asymptomatic period during which infectious organisms can remain in the blood. Falciparum malaria, in particular, can remain in the bloodstream for a long time, even years, as an asymptomatic infection [1]. Because of this, most blood transfusion services have a policy of deferring 'at-risk' donors. Legislation in the United States and the European Community currently states that travellers who have visited malaria-endemic areas may only be accepted as blood donors 6 months after their return to the non-endemic area if they have been free of febrile illnesses and have not taken antimalarial drugs during the 6 month period. Immigrants or visitors from endemic areas may only donate blood 3 years after leaving the area if they have been asymptomatic in the interim.

Although this deferral policy is broadly successful, in many non-endemic countries the rise in the number of donors who fall into the risk categories for deferral is causing a threat to the sufficiency of blood stocks. This threat is compounded by the increasingly mixed population in these countries and the subsequent pressure on blood services to ensure that the donor population reflects the hospital population. As a result, national blood services have to continually recruit new donors to maintain adequate blood supplies. This is not only a costly process, but it can also often be ineffective. An alternative, and possibly more cost-effective approach, is to manage existing donors more effectively through a specific screening strategy - many 'malaria-risk' donors are actually malaria-free, so are excluded unnecessarily ('Table 1'). In England alone, for example, it is estimated that 60,000 potential blood donations a year are excluded because of the risk of malaria infection [2]. The availability of a malaria screening test is a prerequisite for any blood transfusion service considering such a strategy in a country where the disease is not endemic.

Malaria screening tests

The development of a test suitable for screening donated blood for malaria has proved particularly challenging. One of the problems is the fact that it takes just one malarial parasite in donor blood to cause disease in the recipient.

The key criteria for a test suitable for malaria screening are:

- high sensitivity and specificity (sensitive enough to reject all contaminated donations and specific enough to achieve a strong positive predictive value)
- low cost
- suitable for large scale laboratory use
- straightforward to perform (results should not depend heavily on the experience of the individuals performing the test).

A number of different test types have been developed, best divided into those involving direct (antigen) detection and those involving indirect (antibody) detection.

Direct (antigen) methods

Theoretically, standard parasite (antigen) detection tests must be able to detect a single malarial parasite per unit of donor blood to be safe. For this reason they are not ideally suited for screening purposes. Nevertheless, the most sensitive of these methods, polymerase chain reaction (PCR), which relies on the detection of malarial parasite DNA using a linked amplification system, is used for screening in some countries, e.g. Spain [4]. In addition to concerns about the limits of direct detection methods, PCR analysis has the drawback of being expensive, slow (results can take up to 6 hours) and complex to use.

Indirect (antibody) methods

Indirect methods rely on the detection of antibodies to malarial parasites. Because antibodies do not appear in the bloodstream until some days after initial infection and then persist for some months, indirect methods are not suitable for the diagnosis of malaria. They do, however, provide a suitable basis for screening cases of imported malaria. They are less appropriate as a screening tool in countries where malaria is endemic because of the very high incidence of malarial antibody in the population.

There are two main types of malaria antibody detection test: indirect fluorescent antibody tests (IFATs) and enzyme immunoassays (EIAs). In IFATs a serum sample is exposed to multiprot (slides coated with antigen in the form of blood stage Plasmodium species schizonts). Homologous antibody, if present in the serum, attaches to the antigen, forming an antigen-antibody complex. Fluorescein-labelled anti-human antibody is then added, which attaches to the antigen-antibody complex and can be visualised with a fluorescence microscope. Positive samples turn a fluorescent yellow.

IFAT methods, although sensitive and relatively low in cost, have the drawback that they rely heavily on the experience of the individual performing the test, with significant scope for user error.

Enzyme immunoassays typically use plastic microplate wells coated with antigen. Antibodies present in a sample added to the well bind to the antigen and any unreacted material is washed off. Bound antibody is then detected by addition of a mixture of antigens coupled to an enzyme system. After further washing, the presence of enzyme, and hence the presence of malarial antibodies, is revealed by a colour reaction. EIAs have the advantage of being relatively quick (less than 2 hours) and easy to perform; require minimal specialist equipment; and are suitable for large-scale laboratory use. Their sensitivity and specificity depend upon the nature of the antigens used.

Screening strategies

Somewhat surprisingly, the value of malaria screening over and above the current deferral policy is a matter of controversy. Some argue that the existing deferal criteria for 'malarial-risk' blood donors is highly efficient and quite adequate for ensuring the safety of the blood supply [5]. Others take the view that it excludes too many healthy donors and relies on potentially inaccurate travel and immigration histories during the donor-screening process. In practice, much probably depends on the precise
nature of the blood supply and demand issues in individual countries along with the screening strategy proposed. Where a malaria screening test is advocated, it should always be used in conjunction with donor travel and immigration histories to screen only those individuals with a malarial exposure risk.

Experience of the English National Blood Service

The UK has the highest levels of imported malaria in Europe - primarily *P. falciparum*, but also *P. vivax* - with the estimated loss of 60,000 donations a year due to the need to defer ‘at-risk’ donors.

In an attempt to improve the blood supply, malarial antibody screening was introduced in England in 1997. Its use was relatively short-lived, however, as assay performance proved unsatisfactory. The National Transfusion Microbiology Reference Laboratory (NTMRL) was recently tasked with developing a new IFAT method for malarial screening. The resulting in-house assay was evaluated in parallel with a new EIA test (Newmarket Laboratories) in a regional trial testing over 13,000 blood donors with a malaria exposure risk [2].

Of the 13,608 donations tested, 12,427 (91.3%) were negative on both assays and released for issue into the blood supply. The parallel use of both IFAT and EIA proved highly effective in providing reliable information on the relative sensitivities of both methods. The number of ‘EIA+ only’ samples and the low rate of IFAT+/EIA- samples [Table 2], as well as the failure to identify any significant antibody titres in them, indicated that the performance of the EIA alone was sufficient for screening. Use of the IFAT was not considered to offer any additional benefit over use of the EIA test alone in the testing strategy and could therefore be dropped.

These results have allowed the National Blood Transfusion service to reinstate fully malarial antibody screening using the new Malaria EIA test for selected donors across the country. The success of the trial and confidence in the performance of the new EIA will ease the pressure on blood supply services in the UK. Other countries with high cases of imported malaria are already showing interest in using the test to minimise unnecessary deferral of otherwise acceptable donors.

<table>
<thead>
<tr>
<th>Country</th>
<th>Positive test result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>England</td>
<td>5.47%</td>
<td>Kitchen &amp; Lowe, 2003</td>
</tr>
<tr>
<td>Spain</td>
<td>4%</td>
<td>Benito &amp; Rubio, 2001</td>
</tr>
<tr>
<td>New Zealand (Auckland)</td>
<td>1.7%</td>
<td>Davidson <em>et al.</em>, 1999</td>
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Table 1. Percentage ‘malaria risk’ blood donors testing positive for malaria.

<table>
<thead>
<tr>
<th>Test result</th>
<th>Total ‘at-risk’ blood donations</th>
</tr>
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<tbody>
<tr>
<td>IFAT-/EIA-</td>
<td>91.3%</td>
</tr>
<tr>
<td>IFAT+/EIA+</td>
<td>2.36%</td>
</tr>
<tr>
<td>IFAT+/EIA-</td>
<td>2.52%</td>
</tr>
<tr>
<td>IFAT-/EIA+</td>
<td>3.79%</td>
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Table 2. Results from parallel evaluation of IFAT and EIA malaria screening tests.

References

The authors
Colin Knox, Regulatory Affairs Director, Nigel Appleton, R & D Director, Newmarket Laboratories Ltd Lanwades Business park Kentford, Newmarket Suffolk CB8 7PN UK
www.newlabs.co.uk

Figure 1. The Newmarket Laboratories malaria EIA test