The Current Status and Potential Role of Laboratory Testing to Prevent Transfusion-Transmitted Malaria

Clive R. Seed, Alan Kitchen, and Timothy M.E. Davis

Malaria remains a rare but serious complication of transfusion because of the asymptomatic persistence of parasites in some donors. In nonendemic countries, the predominant strategy of deferral or cellular component discard from "risk" donors is effective in minimizing the incidence but is wasteful. In endemic countries where recipients are commonly immune, transfusion strategies focus on chemoprophylaxis for the donor and recipient or ensure that blood collected in highly endemic regions is not transfused to patients from areas of low endemicity. Donors implicated in transfusion-transmitted malaria are predominantly "semi-immune" with very low parasite loads. Their detection by even the most sensitive antigen or polymerase chain reaction (PCR) assays cannot be guaranteed and, in a number of cases, is unlikely because the infectious dose is estimated to be 1 to 10 parasites in a unit of blood. Retrospective analysis of implicated donors has confirmed the presence of high titer antibodies in such individuals. In regions of low immunity, serological assays offer an efficient method to identify such infectious donors. The recent development of enzyme immunoassays (EIAs) with improved sensitivity to *Plasmodium falciparum* and *Plasmodium vivax*, the predominant transfusion threats, has heightened the appeal of serological testing. Although universal serological screening in nonendemic regions is not cost-effective, targeted screening of donors identified at risk by travel-based questioning can significantly reduce wastage through reinstatement. Importantly, transfusion safety does not appear to be compromised by this approach as evidenced by the lack of a documented transmission in France between 1983 and September 2002, where such a strategy has been used since 1976. The development of automated protein microarray–based technology has the potential to further enhance antibody/antigen sensitivity; however, its application to donor screening is likely to be some years off. There is also the potential that pathogen inactivation techniques currently under development to address the bacterial contamination of blood components may also be effective against malaria parasites to make malarial testing redundant or at least reduce its cost/benefit ratio. Nonetheless, there are still significant problems to be solved in respect of validating and licensing these systems. Assuming that they are successfully marketed, their high cost may also impact their cost-effectiveness in comparison with targeted malaria testing strategies already in place in some jurisdictions.

MALARIA IS A protozoan infection that is almost always transmitted by the bite of the female *Anopheles* mosquito. In rare cases, the infection is acquired by the direct inoculation of infected blood such as during transfusion. The first case of transfusion-transmitted malaria (TTM) was reported in 1911.[1] There are a number of reasons why malaria represents a significant transfusion threat. *Plasmodium species* can survive for at least 3 weeks in refrigerated blood. Depending on the number of parasites in the inoculum, the symptoms of malaria may develop days to weeks after transfusion. The patient may not have a history of potential exposure to malaria and will commonly exhibit nonspecific symptoms without the characteristic fever periodicity. A consequent delay in diagnosis can lead to the death of the patient, particularly if *P falciparum* is the species involved.

Accurate data on the incidence of TTM are confounded by underreporting, particularly in endemic regions where recipients frequently have preexisting infection. In nonendemic regions, the reported incidence ranges from 0 to 2 cases per million donations. In a recent comprehensive review of TTM in the United States, the incidence for the period 1993 to 1998 ranged from 0 to 0.18 cases per million units transfused.[2] A similar incidence can be inferred for Australia with the last reported case occurring in 1991.[3] By contrast, the incidence in endemic countries is likely to exceed 50 cases per million donor units.[4] Recent case reports of TTM from the United States,[5] Canada,[6] Switzerland,[7] and the United Kingdom (Dr A. Kitchen, written communication, January 23, 2004) confirm a continuing transfusion threat in nonendemic countries as a consequence of imported malaria.

From the Australian Red Cross Blood Service, Perth, Australia; National Blood Service, North London, UK; and School of Medicine and Pharmacology, University of Western Australia, Fremantle Hospital, Fremantle, Australia.

Address reprint requests to Mr Clive R. Seed, Australian Red Cross Blood Service, 290 Wellington Street, Perth, Western Australia 6000. E-mail: cseed@arcbs.redcross.org.au

0887-7963/05/$ – see front matter

doi:10.1016/j.tmrv.2005.02.004

Despite significant advances in test development for transfusion-transmitted viral infections in the last 3 decades, a reliable, high-throughput blood screening test for malaria has remained elusive. Recent risk modeling studies from developed countries have demonstrated that the residual risk of transfusion-transmitted HIV and hepatitis C virus are less than 1 in 1 million.[8,9] This has refocused efforts to address other nonviral infectious transmission risks including bacterial and parasitic infections, of which malaria is a universal priority. A second important driver for malaria test development stems from the fact that current strategies to reduce the likelihood of transfusion transmission used in nonendemic countries invariably involve discarding blood from "potentially exposed" donors leading to significant wastage. In the current climate of increasingly stringent donor deferral criteria, leading in turn to frequent sufficiency concerns, the wastage associated with such strategies is unacceptable. This review seeks to summarize some current malaria testing strategies used by blood services and explore the recent advances in test development, including serological and nucleic acid testing (NAT), which might be applied to the problem of identification of malaria-infected blood donations.

EPIDEMIOLOGY

Malaria is a protozoan parasitic infection of human beings resulting from infection by 1 or more of the 4 species of the genus *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*). It is 1 of the most common diseases in the world, and more than half of the world's population lives in malaria-endemic areas. These include parts of Asia, Central and South America, Africa, Oceania, and the Caribbean. Each year, reported cases number between 300 and 500 million worldwide, resulting in more than 1 million deaths annually, the majority in young children. The impact of malaria is greatest in sub-Saharan Africa where approximately 85% of all fatal cases occur.[10]

Of the 4 *Plasmodium* species that cause human disease, *P falciparum* is the most serious and is potentially life-threatening if left untreated in nonimmune individuals. It has a wide geographical distribution but is the predominant species in Africa, Papua New Guinea, and parts of Asia. *P vivax* can cause severe symptoms but is rarely fatal. It is the most widely distributed species, being more common in Central America, the Indian subcontinent, and China. It is not found in West Africa. *P falciparum* and *P vivax* infections occur in equivalent numbers in South America, most of Asia, and Oceania. *P malariae* is found much less frequently but has wide distribution, with the greatest number of reported cases in sub-Saharan Africa. *P ovale* is the rarest of the 4 species with the majority of cases occurring in sub-Saharan West Africa. The level of endemicity is seasonal as well as varying between countries and even between different regions of the same country.[11]

PARASITE LIFE CYCLE

In natural human infection, the infected mosquito releases sporozoites into the bloodstream during a blood meal. (Fig 1) Sporozoites invade hepatocytes and develop into hepatic schizonts. Each hepatic schizont can rupture and release 10 000 to 30 000 merozoites into the bloodstream. This hepatic or exoerythrocytic phase of development ranges from 5 to 7 days in the case of *P falciparum* to 14 days in *P malariae* infections. The merozoites invade erythrocytes and develop into schizonts over 48 hours in *P falciparum* and *P vivax*, 50 hours in *P ovale*, and 72 hours in *P malariae* infections. The schizont then ruptures to release between 8 (*P malariae* and *P ovale*) and 16 (*P falciparum* and *P vivax*) merozoites. After a number of replicative erythrocytic cycles, a pyrogenic density of parasites is reached, and symptoms develop. The incubation period can be as short as 7 days for *P falciparum* malaria, is typically around 14 days in *P vivax* and *P ovale* malaria, and is usually at least 1 month in *P malariae* infections. Some infected erythrocytes produce male or female gametocytes rather than schizonts. These sexual forms are ingested by the mosquito during a blood meal. Sexual reproduction within the mosquito leads to the formation of an oocyst. The oocyst ruptures and liberates sporozoites, which subsequently migrate to the salivary glands of the mosquito.

The replication of *P falciparum* in the human host differs from that of the other species. Its merozoites are capable of invading erythrocytes of all ages, not just younger red cells and reticulocytes favored by *P vivax* and *P ovale* or the senescent erythrocytes targeted by *P malariae*. In addition,

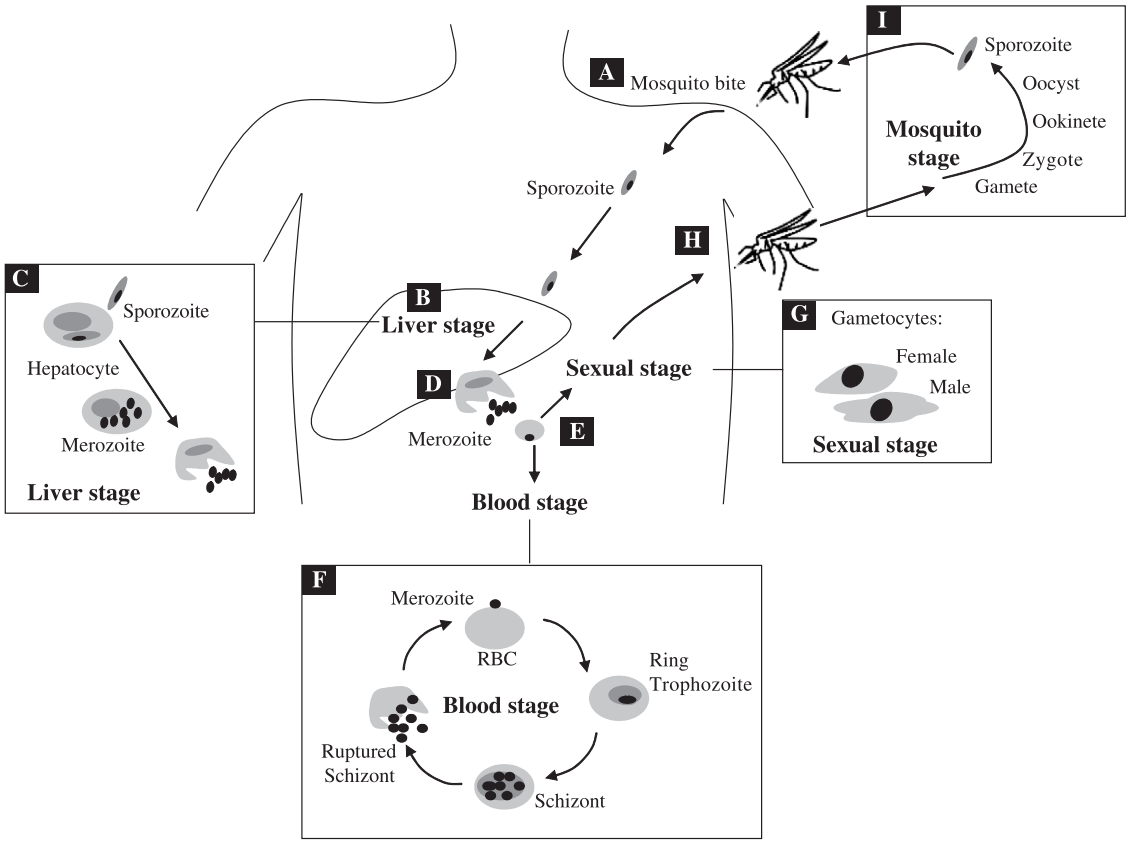

Fig 1. The life cycle of the malaria parasite in human beings. When an infected female *Anopheles* takes a blood meal, malaria infection is introduced by injection of sporozoite-stage parasites into the circulation (A). These sporozoites target and invade liver hepatocytes (B), where extensive intracellular multiplication occurs (C). Eventually, the infected hepatocytes rupture (D) and release merozoite-stage parasites into the circulation. The merozoites invade erythrocytes (E) and develop into ring trophozoite and mature schizonts containing new merozoites (F). Some merozoites differentiate into nondividing male or female gametocytes (G), which can be transmitted to female *Anopheles* mosquitoes as they feed (H). Once inside the mosquito, gametocytes unite in a sexual life cycle and form a zygote in the mosquito midgut (I). Zygote maturation forms thousands of sporozoites that migrate to the mosquito salivary glands. A new life cycle begins when these sporozoites are injected into a human host. Adapted from Hviid.[12]

P falciparum–infected erythrocytes acquire the ability to "sequester" in the microvasculature by sticking to the vascular endothelium. This process of cytoadherence takes place about halfway through the 48-hour life cycle, and so mature forms of *P falciparum* are seldom seen in peripheral blood. Cytoadherence has 2 major effects. First, although the exact pathogenic mechanisms involved remain poorly understood, it can lead to the development of complications such as coma and renal failure. Second, sequestered mature forms are protected from reticuloendothelial clearance, and this can lead to very high parasite densities that are not seen in the 3 other "benign" human malarias and which can themselves contribute to complications. Unlike *P vivax* and *P ovale*, *P falciparum* does not have a hypnozoite form that can persist in the liver and lead to relapses after successful treatment of the primary infection.

Residents of endemic areas subjected to repeated infections gradually develop immune defense mechanisms against malaria. However, unlike viral diseases such as smallpox in which a single infection is sufficient to produce complete immunity to subsequent reinfection, long-term or protective immunity against malaria is rare. Natural immunity takes years to develop and is only maintained by ongoing exposure. Hence, most individuals from endemic regions are in a state of

"semi-immunity," characterized by their capacity to carry low numbers of circulating parasites without clinical manifestations of the disease. Elimination of circulating parasites from "semi-immune" individuals without ongoing reinfection varies; *P falciparum* is generally eliminated within 2 years, *P vivax* and *P ovale* generally within 3 years, but *P malariae* can persist for decades.[13] This long duration of asymptomatic parasitemia is a potential source of TTM.

EPIDEMIOLOGY OF TRANSFUSION-TRANSMITTED MALARIA

P malariae and *P vivax* are the species most frequently associated with TTM. After the original report in 1911, *P vivax* predominated until the 1950s when *P malariae* replaced it as the most common causative species. In the 1970s, an increasing proportion of *P vivax* and *P falciparum* was observed, and the high mortality rate associated with transfusion-transmitted *P falciparum* malaria was reported.[4] In the United Kingdom, for instance, the proportion of malaria caused by *P falciparum* increased from 37% in 1984 to 55% in 1993, mirroring an increase in the proportion of notifications of imported *P falciparum* malaria that were acquired in Africa rather than Asia. The 5 recorded cases in the last 15 years in the United Kingdom have all been attributed to *P falciparum*.[14] (Dr A. Kitchen, written communication, January 23, 2004) Similarly, the US TTM data reveal *P falciparum* as the predominant species during the 1990s, with 6 of 8 fatalities between 1963 and 1999 caused by this species.[2] The donors implicated in TTM are invariably semi-immune with parasite densities below the limit of detection of currently available assays. As a result of the asymptomatic persistence of parasites, transmission of *P malariae* has been documented as long as 53 years[13]; *P vivax*, 27 years[15]; and *P falciparum*, 13 years [15] after last exposure.

Any blood component containing erythrocytes can harbor viable parasites. Although whole blood and red blood cell (RBC) concentrates represent the most common source of TTM, cases involving platelets,[16] leukocytes,[17] fresh frozen plasma,[18] and frozen RBCs [19] have all been reported. Despite the declining infectivity of parasites during storage at 4°C, all species survive for at least a week, although in 1 of the UK transmissions, the implicated donation (whole blood) had been stored at 4°C for 19 days before transfusion (Dr A. Kitchen, written communication, January 23, 2004). The presence of 3% to 4% dextrose is associated with longer parasite viability, with cases of transfusion transmission after 10 and 21 days of storage at 4°C recorded for *P vivax* and *P falciparum*, respectively.[20]

CURRENT MALARIA PREVENTION STRATEGIES

At the time of writing, the authors are unaware of any country having implemented universal blood donor testing for malaria. Targeted testing of "potentially exposed" donors identified by questionnaire has been used by some countries for many years and is now gaining in general popularity in nonendemic countries. A summary of the application of laboratory testing to donor screening is presented in Table 1. Current strategies applied by blood services to prevent transfusion-transmitted infection can be divided into 2 categories according to the prevalence of malaria in the donor population.

Low Prevalence (Nonendemic Donor Population)

In nonendemic areas, the most widely applied approach relies on identifying donors with a risk of malarial exposure through travel or medical history–based questionnaires. This usually involves deferral or cellular (RBC and platelet) product restriction for periods of 12 to 60 months after travel to, or residence in, malaria-endemic countries. This has been highly effective in reducing the incidence in nonendemic regions (eg, North America, Europe, and Australia) but is dependent on both the donor accurately disclosing prior risk and the effective application of this information to direct donor deferral or blood product discard. This system is vulnerable to human error with documented omissions resulting in the release of infectious units and subsequent transmission. For instance, in a review of US transfusion-transmitted malaria, Mungai et al established that 62% of donors implicated in a subsequent transmission would have been deferred from donation if the specified exclusion criteria had been correctly applied.[2] Even if a donor does declare a risk requiring deferral or cellular product discard, errors in applying this information have been recorded. This situation occurred in the last case of TTM in Australia in 1991,[3] as well as a recent case in a US blood donor.[5] A further limitation to travel-based restrictions is

Table 1. International Application of Malarial Donor Screening Tests

Country/Organization	Testing Method	Implementation Status	Reference
ARCBS	Malaria antibody EIA (recombinant pf and pv)	Under consideration	Seed et al[21]
France, National Institute of Health and Medical Research	Malaria IFAT	Implemented	Silvie et al[22]
	Malaria antibody IgG EIA/antigen	Under consideration	
United Kingdom, English National Blood Service	Malaria antibody EIA (recombinant pf and pv)	Implemented	Kitchen et al[23]
New Zealand, Auckland Regional Blood Centre	Malaria antibody IgG EIA	Evaluated, not implemented	Davidson et al[24]
Hong Kong Red Cross Blood Transfusion Service	Malarial antibody EIA	Implemented	W.C. Tsoi, written communication, 4/20/04
Vietnam, Blood Transfusion and Haematology Centre	Malarial PCR assay	Evaluated	Vu et al[25]
Nigeria, University College Hospital of Ibadan	Malaria IFAT	Evaluated	Achidi et al[26]
	Malaria antibody–ELISA	Evaluated	
Venezuela, University Central of Venezuela	Malaria IFAT	Evaluated	Contreras et al[27]
	Malaria antibody IgG–ELISA		
Spain, National Institute of Health	Malaria seminested multiplex PCR	Evaluated	Benito and Rubio[28]
India, Blood Transfusion, Postgraduate Institute of Medical Education and Research	Malaria IFAT	Evaluated	Choudhury et al[29]
	Malaria antibody–ELISA		
	Malaria antigen–monoclonal antibody		

Abbreviations: ARCBS, Australian Red Cross Blood Service; EIA, enzyme immunoassay; pf, *Plasmodium falciparum*; pv, *Plasmodium vivax*; IFAT, immunofluorescent antibody test; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

that the long periods of asymptomatic carriage, particularly with associated with *P malariae*, mean that donors can harbor parasites even after their exclusion period.

At the same time, the use of travel-based restrictions leads to wastage of blood products. For example, Chiodini et al[14] estimated that approximately 40 000 U of red blood cells were discarded annually as a result of the UK National Blood Service strategy. Even in the United States, which has a markedly lower rate of imported malaria, the wastage has been estimated at 50 000 blood donations annually.[30] In an attempt to address this problem, some countries have combined travel-based risk exclusion with serological testing to shorten the restriction period allowing earlier reinstatement of donors.[14,22]

High Prevalence (Endemic Donor Population)

In areas of high endemicity, travel-based restrictions and serological testing are ineffective because of the high level of existing immunity against a background of a limited supply of blood products. Strategies to reduce the incidence of TTM have therefore focused on either provision of antimalarial chemoprophylaxis for donor and recipient, or transfusion policies restricting transfusion of donated blood collected in areas of high endemicity to patients from those high endemicity areas or to those with preexisting immunity. Universal testing of donated blood for malaria parasites has not been feasible because of the lack of an appropriately sensitive and cost-effective test (discussed later). Microscopic examination for malaria parasites using Giemsa-stained blood films and antigen detection by monoclonal antibody have been applied in India[29] and many countries in Africa, whereas in Vietnam, the use of PCR to screen donated blood in place of microscopy has been suggested.[25] Another potential strategy involves direct addition of antimalarial drugs such as chloroquine or quinine directly into the donated unit (cf, gentian violet and *Trypanosoma cruzi*), although the efficacy of this approach has not been assessed accurately.[30] One potential problem with this approach is that antimalarial drugs are stage-specific and often need to be given for several parasite life cycles to ensure cure. A brief exposure

to an antimalarial drug at therapeutic concentrations in a unit of infected whole blood before transfusion and the subsequent dilution and metabolism of the drug when it is administered may not reduce transfusion risk significantly.

LABORATORY TEST METHODS FOR MALARIA

Test methods for malaria can be broadly classified into 2 categories: "direct" and "indirect." Direct methods detect parasite or parasite subcomponents, examples of which include microscopic examination of stained slides (Giemsa, Field, Wright, or acridine orange [AO]–stained films), circulating parasite antigens (histidine-rich protein 2, plasmodial lactate dehydrogenase, or aldolase), or plasmodial DNA (PCR). Indirect methods detect host responses to infection; examples include antimalarial antibodies (indirect immunofluorescent antibody test [IFAT], enzyme immunoassay [EIA]) and iron pigment detection (hemozoin).

Because most tests have been developed for the clinical diagnostic setting where the probability of infection and therefore the index of suspicion are high, application to low-prevalence blood donor populations is problematic. The suitability of each method for donor screening needs to consider several key criteria including the prevalence of and immunity to malaria in the donor population, test sensitivity (in particular, that for *P falciparum*), cost, reliability, speed, and complexity. Of primary consideration for direct tests is sensitivity because it has been established that as few as 10 parasites per unit of red cells are sufficient to transmit infection.[31] In the context of a 250-mL unit of RBCs, a test sensitivity of 0.00004 parasites per microliter of RBCs would be required to identify a potentially infectious donation. In respect of indirect tests, of which serology assays are by far the most common, sensitivity for low-titer antibodies apparent early in infection and specificity in low prevalence populations, as well as antibody recognition of all 4 species, are key considerations.

Direct Methods

Microscopy. The most widely applied diagnostic test for malaria is examination of Giemsa- or Wright-stained thick and thin blood films.[32] Blood can be obtained by finger/earlobe prick or venipuncture. The worldwide application of this method as a "gold standard" diagnostic assay is primarily a result of its ability to allow speciation, quantitation of parasitemia, and assessment of the distribution of parasite forms. These latter 2 functions can help in the assessment of disease severity and sometimes influence choice of therapy. The sensitivity of the method varies between 5 and 500 parasites per microliter of whole blood, depending on the expertise of the microscopist.[33] In experienced hands, sensitivities of between 5 and 50 parasites per microliter can be achieved, but most laboratories achieve a lower sensitivity of around 500 parasites per microliter.[34]

Fluorescence microscopy techniques based on dyes with affinity for parasite nucleic acids have also been applied as diagnostic assays.[35-37] A commonly used dye is AO, which when excited by UV light of the correct wavelength fluoresces strongly. Difficulty in discriminating between fluorescence-stained parasites and other nucleic acid–containing cellular debris has limited the sensitivity of AO techniques to >100 parasites per microliter. Although processing time is reduced over routine microscopy, there is a requirement for special equipment. Species differentiation is often difficult and requires confirmation by alternative methods. For these reasons, fluorescent methods offer little, if any, improvement over standard staining techniques.

Despite their continued application as key diagnostic tests, microscopic techniques have several key limitations which render them inappropriate for universal or targeted donor screening. Specifically, they lack the required sensitivity to detect all infectious units, are too time-consuming (generally requiring an hour or more for preparation and thorough examination), and require significant expertise and specialized equipment when fluorescence methods are used.

Antigen detection. Malarial antigen assays based on detection of histidine-rich protein 2, plasmodial lactate dehydrogenase, or aldolase have been developed. These assays were designed as rapid diagnostic tests using various immunochromatographic techniques to detect antigen in whole blood in patients suspected of having malaria. More recently, EIA-based antigen assays suitable for whole blood or plasma have become available. Assay sensitivities range from 100 to 1000 parasites per microliter dependent on species and method, but they have generally comparable sensitivity to microscopy performed in all but the

most expert of hands.[38] Most rapid diagnostic tests are in a "dipstick" format that can be used with minimal training and provide a result within 10 to 20 minutes. Expense and relative insensitivity have restricted their application as donor screening tests.

The recent development of antigen-based EIAs designed specifically for blood donor screening should address cost and throughput limitations; however, the lack of sensitivity and requirement for more complex processing equipment remain disincentives in endemic countries. In nonendemic donor populations and when combined with antibody detection, antigen assays do appear to offer the potential to improve overall sensitivity. For instance, in France, where serological immunofluorescence (IFAT) assays have been widely applied for targeted screening of exposed donors, a dipstick antigen assay modified for plasma was evaluated in a combination strategy with an immunoglobulin (Ig) G–based antibody EIA.[22] In IFAT-positive sera, 71% were detected by the IgG EIA alone, increasing to 88% if the antigen assay result was also available. The authors concluded that a dual strategy incorporating both antigen and antibody assays may be suitable as a replacement for their current labor-intensive but appropriately sensitive IFAT-based screening strategy. Consistent with this finding, a recent Australian Red Cross Blood Service (ARCBS) study performed on acute sera from blood smear–positive patients found a small but measurable sensitivity advantage for the combined strategy over the antibody EIA alone.[21] A commercial antibody EIA, different from that used in the French study, detected 106 (98%) in 108 acute *P falciparum* malaria cases. The 2 antibody-negative sera had detectable antigen using a commercial antigen EIA, making the sensitivity of the combined strategy 100%.

Plasmodial DNA assays. Detection of plasmodial DNA using NAT techniques has been developed both for diagnostic and donor screening applications. PCR assays have, in particular, demonstrated enhanced sensitivity and specificity over other available diagnostic techniques.[33,39] Because of this, PCR assays have also been evaluated for blood donor screening. In a study of Vietnamese blood donors, PCR was shown to be markedly more sensitive than microscopy, detecting 19 of 30 in comparison to 4 of 30 low-level parasitemic donors, respectively.[25] In a similar Spanish study, a semi-nested PCR assay specific for *P falciparum* applied to potentially exposed donors demonstrated a sensitivity of between 0.004 and 0.04 parasites per microliter and was able to detect microscopy-negative, presumptively infectious donors.[28] The authors advocated combining their PCR assay with donor questioning in a targeted strategy to reduce the deferral period for recent immigrants to Spain from 3 years to 6 months. In a recent multisite evaluation in which ARCBS was a participant, the sensitivity of a commercial blood donor PCR screening assay (RealArt Malaria PCR; Artus-biotech, Germany) was determined to be approximately 1 parasite per microliter when assessed using acute samples from Thailand (W. Bolton, written communication, December 23, 2003).

Despite the marked improvement in the limit of detection of these NAT-based assays over other available techniques, even the most sensitive example (0.004 parasites per microliter) is 100-fold shy of the required sensitivity (0.00004 parasites per microliter) to detect all potentially infectious units. (See Fig 2) NAT is therefore unable to detect individuals who may have been exposed to malaria, are malarial antibody–positive, but who have transient parasitemic episodes. This limitation, along with the complex nature and high comparative cost, makes the current NAT assays unsuitable for universal or targeted blood donor screening at present.

Indirect Methods

Serology. Antibodies to all 4 *Plasmodium* species are produced by virtually all individuals 1 to 14 days after initial infection and are detectable for months to years after parasite clearance.[27,40] A positive result on an antibody-based assay can therefore be indicative of either current clinical or subclinical malaria, or of immunity in parasitemic individuals from endemic areas. Although the latter situation is a limitation when screening for active infection, antibody detection assays have found application both to screen donors at risk for prior exposure in nonendemic areas[14,22,24] as well as to confirm putative infection in asymptomatic non-parasitemic donors implicated in transfusion-transmitted malaria cases.[2]

The utility of any antibody-based screening method is primarily dependent on the underlying antibody prevalence in the donor population. In endemic areas where the proportion of antibody-

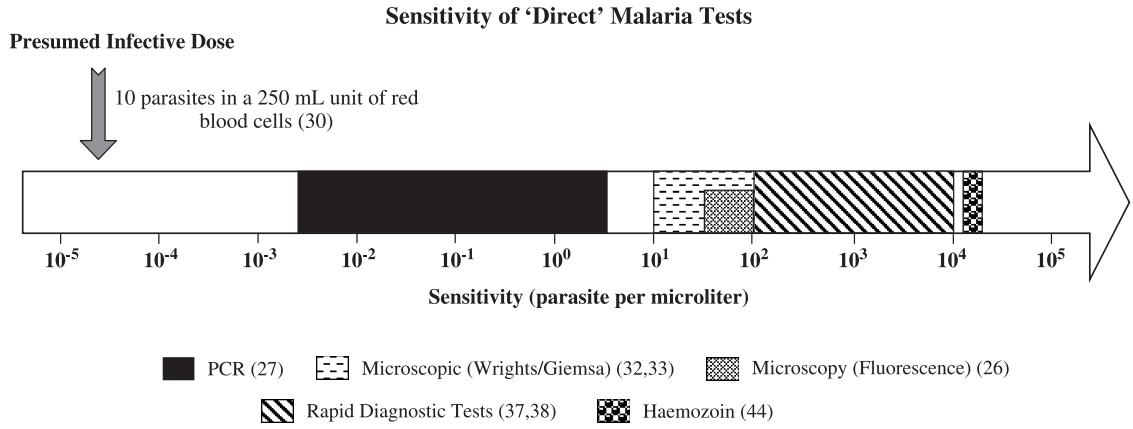

Fig 2. Test sensitivity ranges (parasites per microliter) of "direct" malarial tests.

positive individuals is generally between 20% and 90%,[41] the associated donor loss because of positive results compromises the efficacy of the test for screening purposes. In nonendemic populations, where the rate of antibody-positive donors among those identified as potentially exposed by questioning is only 1% to 2%,[14,24] antibody screening has found favor. In France, for instance, IFAT in combination with travel-based questioning has been used as part of a targeted screening strategy since 1983 and continues currently.[42] Donors returning from malaria-endemic countries are initially deferred for 4 months and then IFAT-tested between 4 months and 3 years with a negative test qualifying the donor for reinstatement. The efficacy of this approach in preventing TTM is supported by the lack of a reported transmission between 1984, when mandatory reporting of transfusion complications was instituted, and September 2002.[22] Despite the excellent sensitivity of IFAT, it has several important limitations. First, the majority of assays are limited to *P falciparum* antibody detection with only limited cross-reactivity for other species, and second, the assay is time-consuming and subjective, requiring significant expertise to ensure reproducibility.

In an attempt to address these limitations, antibody-based EIAs have been developed specifically for blood donor screening. Initially, these assays were restricted to *P falciparum* antibody detection because they were based on antigen from cultured *P falciparum*. Chiodini et al[14] investigated the suitability of an EIA based on native *P falciparum* antigen with specificity for IgG antibody to screen previously exposed UK blood donors. The assay demonstrated excellent antibody sensitivity of 93% in IFAT-positive sera with 1.5% of potentially exposed donors testing positive. Sensitivity for infection in 150 samples from patients with slide-positive malaria was 73% and 52% for *P falciparum* and *P vivax*, respectively. The authors concluded that the assay was sufficiently sensitive provided that the test was performed at least 6 months after the donor's last potential exposure. Their rationale was that this delay would allow sufficient time for a full antibody response to develop. They concluded that the combination of a 6-month restriction on EIA screening combined with the existing travel-based restrictions would provide added security compared with history alone. In support of this contention, they cited 3 cases of transfusion transmission in the United Kingdom that would likely have been interdicted if antibody testing had been in place as an additional safeguard in potentially exposed donors identified by questioning. The benefit of such a policy change was an estimated saving of 40 000 RBCs per annum. Concerns were raised about reliance on a single antigen assay restricted to IgG antibody detection, as well as the low sensitivity for nonfalciparum species, in particular, *P vivax*.[43,44] Despite this, the assay was subsequently implemented at the English National Blood Service in 1997 for targeted screening using a similar approach to that in France. At-risk donors identified by questioning were initially restricted to fractionated plasma–only donations for 6 months. With the exception of

those defined as residents or those who had a history of malaria, donors testing EIA-negative after 6 months were reinstated for full donation. Testing was discontinued in 1998 after the performance of the assay was found to be deteriorating, to the point at which it was considered unwise to continue using it.

The same assay was also evaluated for targeted screening of potentially exposed New Zealand blood donors. Davidson et al[24] found a similar incidence of 1.7% when screening 530 donors identified as having traveled to a malarious area. Sensitivity was evaluated in 11 slide-positive patient sera with 2 (100%) of 2 *P falciparum* patients but only 3 (50%) of 6 *P vivax* patients being detected. Two unspecified cases were also detected but a single mixed *P malariae*/*P vivax* case went undetected. Although the authors acknowledged the sensitivity limitation (50%) in respect of *P vivax*, they concluded that this did not compromise the efficacy of the assay because only 16% of their donors were exposed to this species. Consistent with the UK policy, Davidson et al advocated implementing a dual screening strategy augmenting the existing travel-based identification of potentially exposed donors with serological screening performed at least 6 months after the donor returned from the malarious area. A request to introduce this protocol in Auckland blood donors in 1998 was, however, subsequently rejected by the New Zealand Government, primarily because the EIA was limited to a single antigen, and the sensitivity for nonfalciparum species from cross-reactivity alone was inadequate.

A recent advance in antibody screening for donors has been the development of multiantigen EIAs using recombinant rather than native antigens. Sensitivity for *P falciparum* and *P vivax* has improved markedly over previous single antigen assays. ARCBS has recently assessed 2 such commercially available recombinant assays with one in particular showing high sensitivity. The Newmarket Malarial Ab EIA (Newmarket Laboratories, Newmarket, England) was developed by Newmarket in conjunction with the Parasitology Reference Laboratory, Hospital for Tropical Diseases, London, and the National Transfusion Microbiology Reference Laboratory, National Blood Service, England, and is based on an EIA sandwich format incorporating 4 recombinant antigens to *P falciparum* and *P vivax*.

The performance of the assay was found to superior to IFAT and was considered to be suitable for use for blood screening within the UK transfusion services.[23] Overall, 114 (82.6%) of 138 samples from patients with *P falciparum* and 11 (84.6%) of 13 samples from patients with *P vivax* tested positive; 714 (5.47%) of 13 053 samples from donors identified as "malaria risk," because of residency or travel, were reactive in the EIA.

In the ARCBS study, the assay detected 106 (98%) of 108 and 12 (100%) of 12 of acute samples from slide-positive cases of *P falciparum* and *P vivax*, respectively.[21] On the basis of these and other data, in 2001, the English transfusion service recommenced a screening strategy using this assay in combination with the IFAT and questioning to retrieve the donations from donors with malaria risk.

Existing strategies combining antibody screening and questioning in Europe mandate a 4- to 6-month delay before testing is undertaken. Given this, the sensitivity performance of the newer recombinant assays in acute sera should be considered as "worst case" because this time delay before testing should allow a full antibody response to develop.

Hemozoin. The detection of the hemozoin, a haem malarial breakdown product ingested by monocytes, forms the basis of a novel indirect malarial screening test. Using the property of hemozoin to depolarize laser light, automated hematology analyzers have the capacity to discriminate normal monocytes from those having ingested hemozoin, thereby providing an indication of potential infection. However, sensitivity and specificity at present do not approach conventional detection methods. For example, in 1 study by Wever et al, the sensitivity in a sample of 58 malaria patients diagnosed by conventional methods was limited to only 62%.[45] Although promising because of the possibility of automation of the hemozoin, detection method is currently unsuitable for donor screening based on the lack of sensitivity alone.

Testing Options

Is universal testing feasible or cost-effective? In assessing the merit of the case for universal testing for malaria, the 2 key considerations are the availability of a suitable assay and the incidence of TTM in the recipient population. Regarding a

suitable test, we conclude that, despite significant advances, none currently meets the required criteria for universal screening on the basis of sensitivity alone. If one considers that the most promising direct test (seminested PCR) is still 100-fold short of the required sensitivity to detect all infectious units, in addition to the difficulty of applying such a technically demanding method in endemic countries, the case for universal testing has no justification.

Assuming that the technical limitations were soluble, a recent Canadian study involving 4 different malarial screening strategies indicates that universal screening is not cost-effective. Although universal screening by PCR was the most effective strategy in terms of avoiding transmissions (0.4 per million donors), the relative cost when compared with targeted screening by PCR of donors identified at risk by questioning was estimated to be US$3 972 624 per case averted.[46]

Targeted screening. In nonendemic countries where the risk of TTM is associated with imported malaria, targeted donor testing is a feasible and cost-effective strategy. Serological screening is the test of choice because retrospective analysis of donors implicated in transmissions has identified that such donors are predominantly semi-immune[2,47] with parasitemia below the limit of detection of even the most sensitive assays in the absence of symptoms.[48] Because semi-immune individuals retain high antibody titers,[49-51] they would be excluded as donors by a sensitive antibody assay.

The efficacy and safety of antibody-based targeted screening have been well demonstrated in France with IFAT. The development of recombinant antigen-based malarial antibody EIAs provides the opportunity to further optimize the strategy by addressing some key limitations of IFAT. Specifically, EIAs offer the ability to automate processing as well as removing the requirement for the subjective interpretation that can compromise IFAT result reproducibility. Preliminary studies indicate that the sensitivity of the recombinant EIAs matches or exceeds that of IFAT both for *P falciparum* and *P vivax*, the 2 most significant species in respect of TTM. It is evident from both the United Kingdom and ARCBS data that improvement in sensitivity does not compromise specificity. In fact, in the UK study, the IFAT was more prone to false positives without enhancing sensitivity, the rationale for discontinuing parallel screening in favor of the EIA alone (Kitchen et al, 2004).

The ARCBS study provides further evidence that a combined strategy of identifying risk exposure by questioning linked to subsequent testing is safe as well as cost-effective. Using a mathematical model, the additional risk exposure associated with a reduction from 12 to 6 months (with concurrent antibody testing) in the "fractionated plasma–only" restriction period for donors visiting endemic areas was estimated to be 1 in 6 million (0.006 cases per annum) for *P falciparum*. The authors concluded that when balanced against the saving of an estimated 17 000 RBC U per annum, the additional risk was acceptable and supported implementation of the combined strategy.

Future Considerations

As noted previously, current NAT assays fall well short of the sensitivity requirements to detect all infectious units. Further assay development may address these sensitivity limitations, but this is likely to come at a significant cost premium, therefore negatively impacting the cost/benefit ratio for NAT. Development of protein or DNA microarray technology has the potential to enhance sensitivity for malarial antigens. The ability to multiplex multiple infectious agents on a single microarray in addition to automated processing makes this technology highly cost-effective. Despite the rapid development in the field, its application to blood donor screening is likely to be some way off.[52]

Pathogen inactivation techniques being developed principally to address bacterial contamination of blood components are a potential competitor to malarial testing strategies. Several commercial companies are developing systems capable of inactivating microorganisms including malaria parasites. Successful implementation of such technology has the potential to make testing strategies redundant. Despite their unquestionable potential, there are still several problems requiring resolution including a loss of cellular yield (10%-20%), potential for toxic of mutagenic side effects, and their high cost.[53] These and other limitations have retarded their progress to market with the potential that they may not supersede efficient testing strategies for malaria.

REFERENCES

1. Woolsey G: Transfusion for pernicious anaemia: Two cases. Ann Surg 53:132-135, 1911
2. Mungai M, Tegtmeier G, Chamberland M, et al: Transfusion-transmitted malaria in the United States from 1963 through 1999. N Engl J Med 344:1973-1978, 2001
3. Whyte GS: Therapeutic goods and malaria. Med J Aust 157:439-440, 1992
4. Bruce-Chwatt LJ: Transfusion malaria revisited. Trop Dis Bull 79:827-840, 1982
5. Centers for Disease Control and Prevention: Probable transfusion-transmitted malaria. MMWR Morb Mortal Wkly Rep 52:1075-1076, 2003
6. Slinger R, Giulivi A, Bodie-Collins M, et al: Transfusion-transmitted malaria in Canada. CMAJ 164:377-379, 2001
7. Frey-Wettstein M, Maier A, Markwalder K, et al: A case of transfusion transmitted malaria in Switzerland. Swiss Med Wkly 131:320, 2001
8. Seed CR, Cheng A, Ismay SL, et al: Assessing the accuracy of three viral risk models in predicting the outcome of implementing HIV and HCV NAT donor screening in Australia and the implications for future HBV NAT. Transfusion 42:1365-1372, 2002
9. Glynn SA, Kleinman SH, Wright DJ, et al: International application of the incidence rate/window period model. Transfusion 42:966-972, 2002
10. World Health Organization: The world health report. Geneva, WHO, 2004. Available at www.who.int/whr/2002/en/
11. Miller LH, Good MF, Milon G: Malaria pathogenesis. Science 264:1878-1883, 1994
12. Hviid L: Clinical disease, immunity and protection against *Plasmodium falciparum* malaria in populations living in endemic areas. Expert Rev Mol Med 1998:1-10, 1998
13. Gauzzi M, Grazan S: A relapse of quartan malaria after 53 years latency. Trop Dis Bull 61:11-12, 1964
14. Chiodini PL, Hartley S, Hewitt PE, et al: 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. Vox Sang 73:143-148, 1997
15. Besson P, Robert JF, Reviron J, et al: 2 Cases of transfusional malaria. Attempted prevention combining an indirect immunofluorescence test with clinical selection criteria. Rev Fr Transfus Immunohematol 19:369-373, 1976
16. Garfield MD, Ershler WB, Maki DG: Malaria transmission by platelet concentrate transfusion. JAMA 240:2285-2286, 1978
17. Dover AS, Guinee VF: Malaria transmission by leukocyte component therapy. JAMA 217:1701-1702, 1971
18. Lazner E, Newhouser E: Studies on the transmissibility of malaria by blood transfusions. Am J Med Sci 204: 141-146, 1943
19. Najem GR, Sulzer AJ: Transfusion-induced malaria from an asymptomatic carrier. Transfusion 16:473-476, 1976
20. Boventer K: The behaviour of malaria parasites in preserved blood. Trop Dis Bull 46:799, 1949
21. Seed C, Cheng A, Keller A, et al: Comparison of the efficacy of two malarial antibody enzyme immunoassays for targeted blood donor screening (abstr). Transfusion 44:99A, 2004 (suppl)
22. Silvie O, Thellier M, Rosenheim M, et al: Potential value of *Plasmodium falciparum*–associated antigen and antibody detection for screening of blood donors to prevent transfusion-transmitted malaria. Transfusion 42:357-362, 2002
23. Kitchen AD, Lowe PH, Lalloo K, et al: Evaluation of a malarial antibody assay for use in the screening of blood and tissue products for clinical use. Vox Sang 87:150-155, 2004
24. Davidson N, Woodfield G, Henry S: Malarial antibodies in Auckland blood donors. N Z Med J 112:181-183, 1999
25. Vu TT, Tran VB, Phan NT, et al: Screening donor blood for malaria by polymerase chain reaction. Trans R Soc Trop Med Hyg 89:44-47, 1995
26. Achidi EA, Perlmann H, Berzins K: Asymptomatic malaria parasitaemia and seroreactivities to *Plasmodium falciparum* antigens in blood donors from Ibadan, south-western Nigeria. Ann Trop Med Parasitol 89:601-610, 1995
27. Contreras CE, Pance A, Marcano N, et al: Detection of specific antibodies to *Plasmodium falciparum* in blood bank donors from malaria-endemic and non-endemic areas of Venezuela. Am J Trop Med Hyg 60:948-953, 1999
28. Benito A, Rubio JM: Usefulness of seminested polymerase chain reaction for screening blood donors at risk for malaria in Spain (letter). Emerg Infect Dis 7:1068, 2001
29. Choudhury N, Jolly JG, Mahajan RC, et al: Malaria screening to prevent transmission by transfusion: An evaluation of techniques. Med Lab Sci 48:206-211, 1991
30. Katz LM: Transfusion induced malaria, in Brecher ME (ed): Bacterial and parasitic contamination of blood products. Bethesda, AABB Press, 2003, pp 127-151
31. Boyd M (ed): Epidemiology of malaria. Philidelphia, WB Saunders, 1949
32. Warhurst DC, Williams JE: ACP broadsheet no 148. July 1996. Laboratory diagnosis of malaria. J Clin Pathol 49: 533-538, 1996
33. Moody A: Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev 15:66-78, 2002
34. Milne LM, Kyi MS, Chiodini PL, et al: Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. J Clin Pathol 47:740-742, 1994
35. Baird JK, Purnomo, Jones TR: Diagnosis of malaria in the field by fluorescence microscopy of QBC capillary tubes. Trans R Soc Trop Med Hyg 86:3-5, 1992
36. Benito A, Roche J, Molina R, et al: Application and evaluation of QBC malaria diagnosis in a holoendemic area. Appl Parasitol 35:266-272, 1994
37. Bosch I, Bracho C, Perez HA: Diagnosis of malaria by acridine orange fluorescent microscopy in an endemic area of Venezuela. Mem Inst Oswaldo Cruz 91:83-86, 1996
38. Huong NM, Davis TM, Hewitt S, et al: Comparison of three antigen detection methods for diagnosis and therapeutic monitoring of malaria: A field study from southern Vietnam. Trop Med Int Health 7:304-308, 2002
39. Playford EG, Walker J: Evaluation of the ICT malaria P.f/P.v and the OptiMal rapid diagnostic tests for malaria in febrile returned travellers. J Clin Microbiol 40:4166-4171, 2002
40. Park CG, Chwae YJ, Kim JI, et al: Serologic responses of Korean soldiers serving in malaria-endemic areas during a

recent outbreak of *Plasmodium vivax*. Am J Trop Med Hyg 62:720-725, 2000

41. Akinboye DO, Ogunrinade AF: Malaria and loaisis among blood donors at Ibadan, Nigeria. Trans R Soc Trop Med Hyg 81:398-399, 1987

42. Courtois F, Farragi M, Richard-Lenoble D, et al: Trial routine detection of malaria: Experimentation of 1500 donors. Rev Fr Transfus Immunohematol 19:357-361, 1976

43. Mertens G, Vervoort T, Heylen S, et al: Malaria antibody ELISA insufficiently sensitive for blood donor screening. Vox Sang 77:237-238, 1999

44. Gillon J: Introduction of malaria antibody ELISA. Vox Sang 75:80-81, 1998

45. Wever PC, Henskens YM, Kager PA, et al: Detection of imported malaria with the Cell-Dyn 4000 hematology analyzer. J Clin Microbiol 40:4729-4731, 2002

46. Shehata N, Kohli M, Detsky A: The cost-effectiveness of screening blood donors for malaria by PCR. Transfusion 44:217-228, 2004

47. MMWR: Probable transfusion-transmitted malaria—Houston, Texas 2003. MMWR Morb Mortal Wkly Rep 52: 1075-1076, 2004

48. Ramasamy R: Molecular basis for evasion of host immunity and pathogenesis in malaria. Biochim Biophys Acta 1406:10-27, 1998

49. Draper CC, Sirr SS: Serological investigations in retrospective diagnosis of malaria. BMJ 280:1575-1576, 1980

50. Bruce-Chwatt LJ, Dodge JS, Draper CC, et al: Sero-epidemiological studies on population groups previously exposed to malaria. Lancet 1:512-515, 1972

51. Collins WE, Skinner JC, Jeffery GM: Studies on the persistence of malarial antibody response. Am J Epidemiol 87:592-598, 1968

52. Petrik J: Microarray technology: The future of blood testing? Vox Sang 80:1-11, 2001

53. Alter HJ: Emerging, re-emerging and submerging infectious threats to the blood supply (abstract). Vox Sang 87:56, 2004 (Suppl 2)

Update

Transfusion Medicine Reviews
Volume 19, Issue 4, October 2005, Page 325

DOI: https://doi.org/10.1016/j.tmrv.2005.07.001

Erratum

Clive R. Seed

IN THE JULY 2005 issue of *Transfusion Medicine Reviews* in the article "The Current Status and Potential Role of Laboratory Testing to Prevent Transfusion—Transmitted Malaria" by Seed et al (19:229-240), the references cited in Fig 2 on page 236 were misnumbered. The corrected figure appears below.

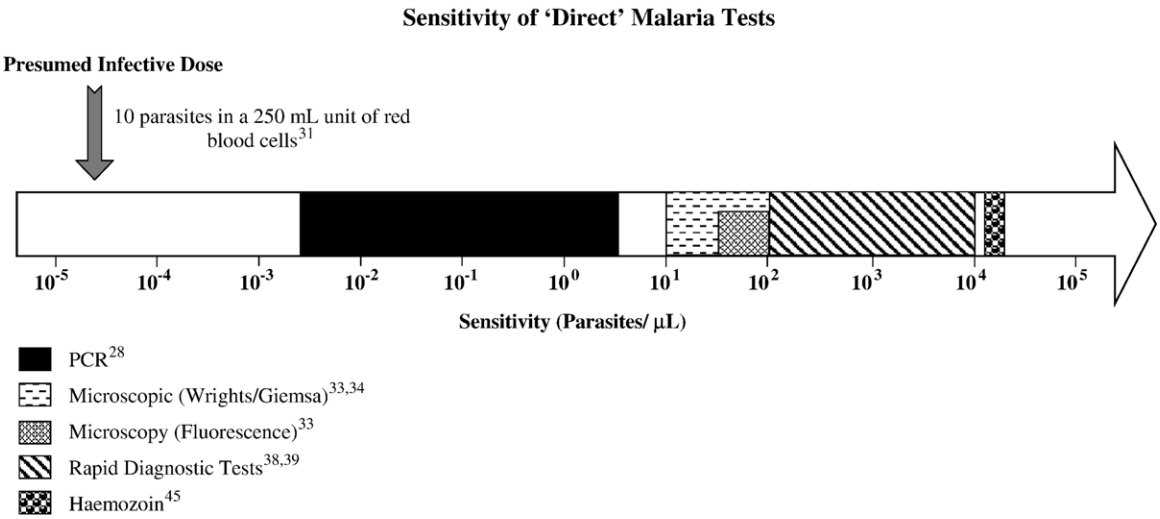

Fig 2. Test sensitivity ranges (parasites per microliter) of "direct" malarial tests.

Doi of original article:10.1016/j.tmrv.2005.02.004

0887-7963/05/$– see front matter

doi:10.1016/j.tmrv.2005.07.001