



Screening at risk blood samples for prevention of transfusion transmitted Malaria



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Objectives:

- To develop a reliable and malaria specific serology test (Antibody detection ELISA) uniformly applicable to all blood banks world wide.
- To provide a reliable testing tool for conferring safety to the donated blood units.
- To identify prospective asymptomatic donors carrying malaria infection.
- To provide a highly sensitive testing system for detecting residual infection with four human malaria parasite species

How important is malaria testing in blood bank operations

- Several types of infectious organism can be transmitted through blood transfusion. For example, viruses (especially Hepatitis C, B, HTLV-VI and- II and HIV), prions (vCJD), bacteria (risk is 50 to 250 higher than those of viral infections) and parasites (malaria, Chagas, Babesia).
- All four species of human malaria parasites are known to be transmitted through blood (whole blood, platelets, leukocytes, and fresh plasma) (TTM).
- The risk of acquiring malaria via transfusion of blood components is extremely low in non-endemic countries such as Australia, USA, and UK. The risk of TTM in the USA has been estimated at 0.25 cases/million donor units. In contrast, the risk in endemic countries may be more than 50 cases/million donor units.
- However there is an increased demand for blood due to increased frequency of transfusion during surgical operations and for treatment of hemophilia. TTM is an emerging global problem and has been reported in several countries such as USA, UK, Canada, Switzerland, and Saudi Arabia.
- The Australian Red Cross Blood Service offers safe blood for transfusion. In many other countries, the Red Cross is solely responsible for providing safe blood for transfusion. "Hemovigilance" is the integral part of the blood program and is operational in EU countries.
- Currently blood banks in USA exclude donors based on travel history. Even then, TTM cases have occurred within the US blood bank systems. Testing at risk samples for TTM is important. The FDA insists a "zero-risk blood supply". The magnitude of the complexity is reflected in the high rate of errors and accidents reported to FDA as results of post-donation information having to do with travel to malaria endemic regions.
- TTM occurs as a consequence of transfusion of infected donor sample. Unless tested, the donor may not be aware of carrying infection. Testing is therefore critical. Serology testing is as PCR (semi nested, Tackman) although highly sensitive, do not completely guarantee a safe blood supply (www.inmpact-malaria.com).
- A serology assay for detecting all four species of human malaria is critically required.

Risk and safety:

Increased tourism into malaria transmission countries and cross continental migration bring in endemic malaria infection into the susceptible population. The donors from such a population invariably are at risk in transmitting infection through their donated blood units.

Non-endemic: In OECD and EU countries, malaria is not endemic. Blood Banks operate under specific regulations by selecting the donors with reliable questionnaires. **Deferral policy is well established.** Screening of infectious agents is the top most priority for providing safety. Malaria antibody testing fits very well. **Specific malaria antibody** is a reliable indicator for deferral of donors.

USA: Donors are excluded based on their travel history and origin for up to 3 years. Malaria is a minor risk of transfusion and therefore no malaria testing is done. So far 93 cases of TTM reported in 1963-1999. Malaria deferrals total around 50,000 donations annually (2.9% of all deferrals).

Endemic: In endemic countries, donors are invariably exposed to infection with a higher % of people showing antibodies. The best strategy is to detect both malaria specific antibody and reconfirm infection by malaria antigen ELISA.

Chagas, Leishmania, and Treponema infections are potentially dangerous and can be transmittable through blood transfusion.

Fig 1. Mixed Plasmodium infection

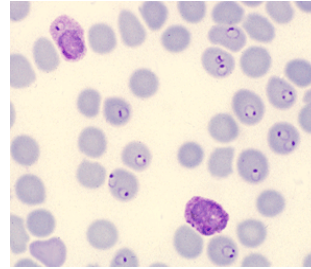
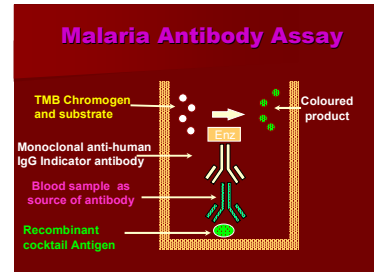


Fig 2. Antibody detection ELISA



Box 1: Malaria parasites in blood samples

[The relative risk of infection varies depending upon the incriminating species and longevity of its survival in asymptomatic blood donors]

P. falciparum: highly virulent species, survive in prospective donors for a year or so. The blood stages survive for 183 days freezing without loss of infectivity.

P. vivax: can survive in prospective donors for 3 years or so.

P. malariae: persists as asymptomatic infection for 25 years or more. Serious transfusion risk (27% TTM in USA).

P. ovale: prospective donors from Africa/Asia typically show asymptomatic infection with latent stages in liver. It can relapse as late as 255 days.



Fig 3: Dextrose is being used as preservative for blood; malaria parasites can remain viable for over 10 days.

Performance of PAN Malaria IgG antibody ELISA

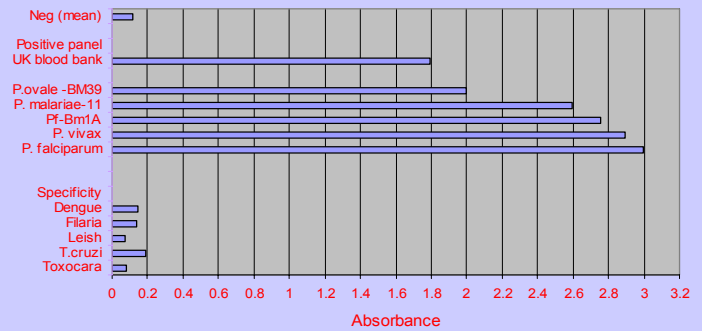


Fig 4: Initially, we constructed an ELISA test for the detection of anti-malaria specific IgG antibodies specific to the four human malaria species in blood samples.

Attributes of Pan Malaria Antibody ELISA:

- This ELISA is based upon a cocktail recombinant antigen and therefore the assay is highly specific.
- Anti-human monoclonal IgG - HRP conjugate is used as marker to overcome a non-specific reaction.
- TMB is employed as a substrate to increase the sensitivity of the assay.

Observation:

- It was found to be sensitive in detecting anti-malaria IgG antibodies against *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* in blood samples. [IgM antibodies are not considered relevant because of its transient nature]
- It showed minimal reactivity with other sera samples.
- This assay is found to be highly specific and no false positivity seen with other (*Toxocara*, *T. cruzi*, *Leishmania*, *Filaria* and *Dengue*) infections.
- The kit is designed for automated system and can be used in any blood banks worldwide.

We evaluated the PAN- kit in different blood banks. Data from Venezuela and from Philippines are described here.

Reactivity of donor samples from Blood Bank Puerto Ayacucho (Endemic for mixed Pf and Pv infection)

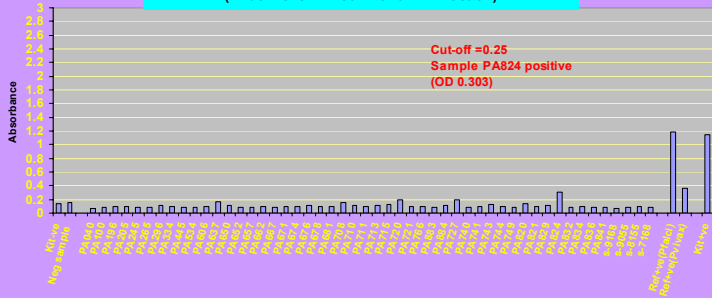


Fig 5: Data from Blood Banks in Venezuela: Altogether n=761 blood samples were tested in four blood banks in collaboration with Dr Carmen Contreras. A cut-off (OD = 0.25) was developed.

Blood bank - Caracas: non endemic n=196 samples tested at Venezuela; n=51 samples re-tested at Cellabs.
Blood bank - Cumana: Sucre state; endemic for P. vivax; n=192 samples tested; retested n=50 samples at Cellabs.
Blood bank - Puerto Ayacucho, Amazonas state: endemic for Pf+Pv; n=186 samples; re-tested n=43 samples at Cellabs.
Blood bank - Bolivar state, endemic for Pf+Pv; n=187 samples; re-tested n=50 samples at Cellabs.
Data from Puerto Ayacucho is shown here. A cut off of OD 0.25 was determined to be suitable for Venezuelan blood bank samples. One sample was found to be positive in Puerto Ayacucho blood bank.

Reactivity of endemic blood bank samples in PAN Malaria IgG CELISA [Data analysis of n=204 samples]

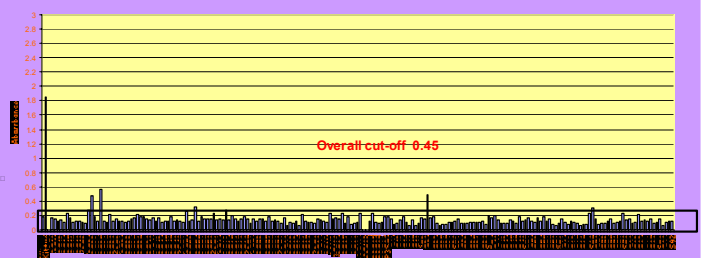


Fig 6: Data from Blood Banks in Philippines: We evaluated the PAN Malaria Antibody ELISA kit at the Philippine National Red Cross Blood Bank (Manila) in collaboration with Dr Marquez and Dr Bonifacio; tested n=204 samples. Developed a cut off (OD=0.450) for reliable identification of positive donor samples. Three samples out of n=204 were found to be reactive and fell in the zone of positivity.

Conclusions

- A Pan Malaria IgG Antibody detection ELISA was developed to screen at-risk blood samples for the prevention of TTM. This test has detected anti-malarial antibodies against four human parasites (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*).
- This kit was evaluated in the blood banks located in Venezuela and in the Philippines. Only a few reactive blood samples were detected which were excluded. Based on this test, a large percentage of donated samples were found to be safe for transfusion.
- Certain criteria should be followed for reliable identification of a positive donor sample. A cut-off absorbance should be developed at individual blood banks based on the local population. A cut-off value may vary between the blood banks due to the nature of donor population.
- Samples from heavily infected or recently infected donors are expected to react strongly (i.e.>OD 1.0) in PAN-ELISA test. Such a highly reactive sample should be excluded. Any sample showing OD between 0.5 and 1.0 is regarded as low infection. This may be from a past infection. Any reactive sample above the cut-off value should be excluded and investigated further.
- This kit was found to be useful for screening the donated blood samples which guarantee the safety of blood supply for transfusion.