Malaria Parasite Diagnostics

One of the most pronounced problems in controlling the morbidity and mortality caused by malaria is the limited access to effective diagnosis and treatment in areas where malaria is endemic. The most widely used routine method of microscopy needs laboratory infrastructure and expertise and is labour intensive. Therefore, development of rapid, sensitive and specific diagnostic tests to diagnose malaria is of paramount importance. NIMR has been making efforts to develop simple methods and recently, the Institute has been identified as the Referral Centre, and commercially developed diagnostic kits are referred from the Office of the Drugs Controller General of India for evaluation before marketing.

Detection of *Plasmodium vivax* in Human Blood using Synthetic DNA Probe

An oligonucleotide based on 19 tandem repeats of a nonapeptide published earlier was synthesized and an assay developed to detect *P. vivax* parasites in infected blood (Roy *et al* 1987). A 21 mer oligonucleotide representing repetitive sequences in genes coding for CS protein of *P. falciparum* was synthesized for comparison of specificity and cross-reactivity. The oligos were radio-labelled and tested as probe in DNA-DNA hybridization assay on nitrocellulose filters. These showed desirable specificity and the vivax probe was used in a simple dot blot assay for detecting parasite in patient's blood. Hybridization with 5 µl of *P. vivax* positive blood from patients spotted on nitrocellulose after lysis gave very strong signal with *Pv* probe but not with *Pf* probe. The results showed that synthetic DNA probe based on repetitive sequences can specifically discriminate *P. falciparum* from *P. vivax*. The importance of correct diagnosis for administration of radical treatment and interruption of transmission can not be over emphasized. Where rapid screening of a large number of samples is necessary, DNA probes will provide desirable speed, accuracy and sensitivity.

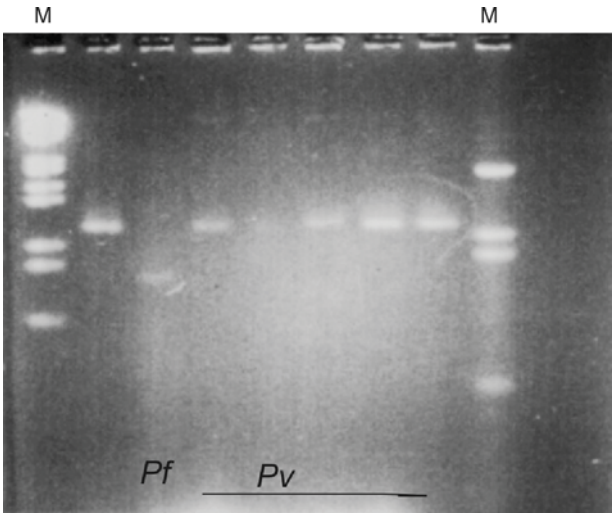

Fig. 1: PCR based diagnosis

PCR based Diagnostics

Simple PCR assay using primers derived from a highly repetitive DNA fragment was employed to amplify DNA fragment from dried blood spots on filter paper (Whatman 3 mm) (Fig. 1). Observed positivity of 97.1% for correct results (both positive and negative) using blood spots on filter paper suggests that the simple PCR assay can be used for the diagnosis of *P. falciparum* and *P. vivax*, the two human malaria parasite species commonly prevalent in our country. Sensitivity (97.6% for *P. falciparum* and 88% for *P. vivax*) and specificity (100% for both) is similar to the results reported by other diagnostic techniques. The study shows the feasibility of using field collected blood spots for the identification of malaria infections.

Production of Monoclonal Antibodies against Erythrocytic Stages of *P. vivax*

Twenty monoclonal antibodies, IgG1 type were raised against erythrocytic stages of *P. vivax*. Antibodies reacted with erythrocytic stages of *P. vivax* clinical isolates by indirect immunofluorescence assay. One clone Mab1B3 was studied for antigenic characterization. Antibody secreted by this clone showed its reactivity in inhibition ELISA when tested with blood samples collected from *P. vivax* patients (Fig. 2). This antibody showed the detecting ability in isolates having > 0.01% parasitaemia. By immunoblotting, this monoclonal antibody reacted

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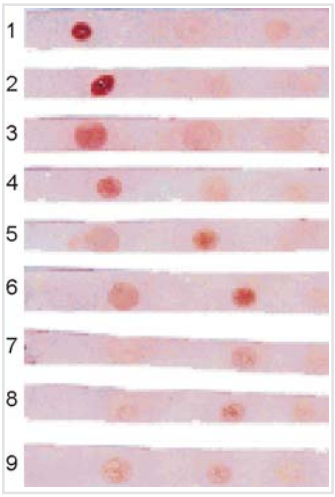

Fig. 2: Dipstick assay

with nearly 42 kDa protein of *P. vivax* parasite collected from different geographic locations of the country. An additional protein of nearly 65 kDa was also recognized by this antibody in some of the isolates collected from Rourkela, Chennai, Bhopal, Ghaziabad and Delhi. The epitope recognized by the antibody from crude extracts of erythrocytic stages of *P. vivax* might be common in these proteins. N-terminal sequencing of the protein identified by this clone produced significant alignments with one *P. falciparum* erythrocyte membrane associated antigen. Enzyme (HRPO)-antibody conjugate was prepared to develop a Dot blot/Dipstick ELISA for the detection of parasite in the patient's blood. The assay is to be evaluated in the field.

Immunodiagnostic Assay of *P. falciparum* Infection using a Glycophospholipid Antigen and Laser Immunoassay

Diagnostic potential of the GPL antigen was assessed by laser light scattering immunoassay (LIA) and ELISA methods. Immunoreactivity and specificity of the GPL antigen is compared with another previously reported RESA derived synthetic peptide antigen. We developed an assay using GPL coated latex beads which could be an excellent diagnostic method for *P. falciparum* infection. Agglutination assay is the simplest and cheapest immunoassay but has not been employed because of its low sensitivity. As the antigen-antibody complexes are often microscopic and not visible to the naked eye, laser immunoassay (LIA) based upon laser light scattering can detect microscopic agglutinates and can raise the sensitivity of agglutination assay enormously. LIA for malaria (Bhakat *et al* 1999) was developed which is as sensitive as ELISA but much simpler in principle and practice. Specific diagnosis of *P. falciparum* infection by LIA using a new glycophospholipid antigen isolated from *P. falciparum* culture supernatant was developed. The chemical composition of the antigen as analysed by HPLC, TLC and GLC showed it to be a glycophospholipid (GPL) with galactose, glucose, mannose and xylose as sugar residues (Mya *et al* 2001). GPL antigen was found to be very specific and could clearly discriminate between *P. falciparum* and *P. vivax* infections in clinically identified patients both from India and Myanmar (Mya *et al* 2002). Malaria diagnostic immunosensor method has been developed recently (Mya *et al* 2002). Specificity of the GPL antigen was also shown by LIA for serum from non-malarial patients. Out of forty finger prick blood samples collected on filter discs, sixteen samples each were from *Pf* and *Pv* positive cases, and eight sera from healthy individuals included as negative control. The results showed that anti GPL titers for *Pf* and *Pv* cases were about 10-fold and 4-fold higher than that of control sera. Studies are in progress to develop a cost-effective and sensitive LIA using glycolipid antigen.

Evaluation of Rapid Diagnostic Kits

In recent years, rapid antigen capture assays based on the detection of *Pf*HRP-2 antigen in peripheral blood for *P. falciparum* have been

Table 9. Evaluation of diagnostic kits

Name	Manufacturer	% Sensitivity/Specificity
ParaSight-F	Becton Dickinson, U.S.A.	93/92.4
ICT *Pf*/*Pv*	Becton Dickinson, U.S.A.	96/93.1 (*Pf*) 75.5/99 (*Pv*)
Rapid Test Malaria	Quoram Diagnostics, Canada	100/98.3
Pf Check-1	Veda Lab., France	87.7/98.9
Determine™ Malaria *Pf*	Dainabot Co., Japan	96.5/87.2
ACCU Stat Malaria	Millennium Bio-Technology Inc., USA	86.9/90.3
ParaCheck	Orchid Biomed. Systems (Goa)	95.8/85.7
OptiMAL	DiaMed, Switzerland	92.2/99.3 (*Pf*) 94.5/98.2 (*Pv*)
ParaHIT f	SPAN	90.9/91.4
First Response Malaria	PMC Ltd	96/95 (*Pf*)
Combo test		83/94 (Non *Pf*)

developed. The dipstick format kits for the detection of malaria antigens were compared with conventional microscopy for the detection of *P. falciparum* malaria. The overall sensitivity and specificity of these kits were more than 90% (Table 9). The advantages were: (i) less time consuming; and (ii) expertise and infrastructure not required. However, the most important limiting factor of these kits is the persistence of HRP-2 even after parasite clearance thereby making monitoring of therapeutic response difficult (Kumar *et al* 1996; Singh *et al* 1997; Valecha *et al* 1998; Singh and Valecha 2000; Ghosh *et al* 2000).

A kit—ICT (*Pf/Pv*) based on the same principle of detection of HRP-2 antigen for *Pf* and a panmalarial antigen for *P. vivax* was also evaluated. The sensitivity and specificity were 74 and 99.1% respectively for *P. vivax* while these were 96% for *P. falciparum*. OptiMAL, a test utilizing monoclonal antibodies against metabolic enzyme of parasite lactate dehydrogenase (LDH) has also been evaluated. This test does not have the limitation of persistence of antigen. The sensitivity of the test for *Pf* and *Pv* was 92.2 and 94.5% respectively. The test becomes negative in parallel with parasite clearance. Based on the data generated by NIMR most of these kits have been registered and marketed.

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