A HIGHLY SENSITIVE, NESTED POLYMERASE CHAIN REACTION BASED METHOD USING SIMPLE DNA EXTRACTION TO DETECT MALARIA SPOROZOITES IN MOSQUITOS

I Vythilingam[1], K Nitiavathy[1], P Yi[2], B Bakotee[3], B Hugo[3], Balbir Singh[4], RA Wirtz[5] and K Palmer[6]

[1]Institute for Medical Research, Jalan Pahang, Kuala Lumpur 50588; [2]National Malaria Center, Phnom Penh, Cambodia; [3]Solomon Islands Medical Research Institute, Honiara; [4]Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Kota Samarahan; [5]CDC Atlanta, USA; [6]WHO Solomon Islands

Abstract. Dried *Anopheles farauti* mosquitos caught in Solomon Islands in 1990 were examined for malaria sporozoites by ELISA and nested polymerase chain reaction (PCR). Only heads and thoraxes were used. *Plasmodium* genus-specific nested PCR amplifications were carried out on all samples. Of the 402 pools of mosquitos that were processed, 30 were positive for malaria. Nest 1 products of positive samples were subjected to further PCR amplifications with species-specific primers for *P. falciparum* and *P. vivax*. Twenty pools were positive for *P. vivax* by PCR while only 7 were positive by ELISA. For *P. falciparum* 2 pools were positive by both ELISA and PCR, and one of these was a pool which was positive for *P. vivax* by PCR and ELISA. Thus the sensitivity of PCR for *P. vivax* was 100% while the specificity was 96.7%. For *P. falciparum* the sensitivity and specificity were 100%. The PCR technique is highly sensitive and can be used on dried mosquitos which makes it a valuable tool for determining sporozoite rates of mosquitos, even in remote areas.

INTRODUCTION

Malaria is still endemic in some tropical countries in the world. Evaluation of malaria campaigns is based not only on detection of parasites in patients but also on monitoring the infection of mosquito populations. Traditionally this has involved the dissection and microscopic examination of hundreds of mosquitos which is time consuming and labor intensive. With the advent of monoclonal antibodies specific to circumsporozoite (CS) antigens, an enzyme linked immunosorbent assay (ELISA), has been developed and shown to be a useful tool for the detection of *P. falciparum* and *P. vivax* in the mosquito vectors (Burkot *et al*, 1984; Wirtz *et al*, 1985).

An ELISA kit is now available and field evaluations demonstrated excellent correlation between ELISA positivity and salivary gland infection rates assessed by dissection (Wirtz *et al*, 1987). However ELISA fails to detect immature sporozoites present in oocystes (Beier *et al*, 1987) and it has been reported that circumsporozoite (CS) proteins in excess of those incorporated in sporozoites can be found in infected mosquitos (Boulanger *et al*, 1988) resulting in sporozoite number being overestimated.

As an alternative to ELISA, attention has been focused upon the development of diagnostic methods based on deoxyribonucleic acid (DNA) probes and polymerase chain reaction (PCR) for detection of parasites in mosquitos. The first demonstration of the application of DNA probes for diagnosis of the malaria parasite *P. falciparum* was by Franzen *et al* (1984). Following this technique other DNA probes for the detection of *P. falciparum* have since been developed (Oquendo *et al*, 1986; Baker *et al*, 1989; Zolg *et al*, 1987). Although DNA probes can be species specific and allow a large number of samples to be identified, sensitivity has been limited. Over 1,000 sporozoites per mosquito are required for reliable detection even with highly repetitive DNA probes (Delves *et al*, 1989).

The most recent and exciting development in the field of recombinant DNA technology has been the polymerase chain reaction (PCR) (Mullis and Faloona, 1987). By using PCR, it is possible to synthesize *in vitro*, millions of copies of a specific target DNA sequence. It has been discovered that there are some regions of the sequences coding ssrRNA are specific to the parasite species from which they are derived (Walters and McCutchan, 1989; Goman *et al*, 1991). These regions were put to use for the designing of primers suitable for use in PCR amplification technique.

Snounou *et al* (1993) designed nested PCR which they felt was more sensitive. A PCR was