# MOLECULAR DIAGNOSTICS OF PLASMODIUM PARASITES

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#### ABSTRACT

Malaria is a major public health problem in tropical and subtropical areas, especially in Southeast Asia region, caused by any of five species of Plasmodium (P. falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi). It still remains a leading cause of morbidity and mortality worldwide. The aim of this study was to design new molecular markers for identification of Plasmodium spp. and compare the parasite species identification using the molecular methods (nested PCR and new multiplex PCR) and microscopy. Blood samples on filter papers were subject to conventional PCR methods using primers designed by us in multiplex PCR and compared with primers of nested PCR of Singh et al. (1999; 2004), as well as with microscopic identification. Of the 109 samples identified as malaria positive by microscopic examination, 15 samples were positive for P. falciparum, 14 for P. vivax, six for P. knowlesi, 72 for P. malariae, two for mixed infection of P. falciparum/P. malariae and 20 which serve as negative controls. Both multiplex PCR and nested PCR detected 12 of P. falciparum single infections. For P. vivax, nine were detected by multiplex and 12 detected by nested PCR. For 72 P. malariae cases, nested PCR detected two as P. falciparum, 57 as P. knowlesi, seven as P. malariae and six negative. However, multiplex PCR identified two as P. falciparum, 57 as P. knowlesi, ten as P. malariae and three negative. For 28 samples which had been identified as P. falciparum, P. malariae and P. vivax by microscopy, they were identified as negative by both multiplex PCR and nested PCR. This shows multiplex PCR is highly sensitive and specific. The multiplex PCR assay is faster than nested PCR and could be used as an alternative molecular diagnosis for the identification and detection of all Plasmodium spp. as it has shorter the time required for screening a large number of samples.

