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Title of Project:

MOLECULAR DIAGNOSTIC METHOD FOR RAPID AND ACCURATE IDENTIFICATION OF MALARIA PARASITE SPECIES

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EXECUTIVE SUMMARY OF RESEARCH

Four of the five malaria parasite species infecting humans have been recorded in Malaysia (*Plasmodium vivax*, *P. falciparum*, *P. malariae* and *P. knowlesi*) with *P. knowlesi* most prevalent in Sabah. Although the fifth species *P. ovale* is reported in Malaysia occasionally, it is only an imported species brought in by an infected visitor.

Plasmodium species are identified mainly by microcopic examination of blood film on glass slides, using mainly the ring stage of the parasite within the red blood cells as a distinguishing feature. This can be done in most hospitals, but the accuracy of the method depends highly on the skill of the technician/ doctor and idetification errors have been recorded.

A molecular method of species identification using DNA of Singh *et al.* (1999) has become popular. However this method involves two stages of PCR protocol and takes consderable time. Using this method, various investigators have shown that errors have been previously made in the identification of *Plasmodium* based on microscopic examination alone. For example, *P. vivax* and *P. malariae* had been misidentified as *P. falciparum* by microscopy, almost all *P. knowlesi* misidentified as *P. malariae* or *Plasmodium vivax* or *P. falciparum* (Singh et al., 1999)

In our research, we also used a molecular method, but we used four different genes, and various DNA markers which could provide more accurate and faster identification, with lower error rates. Our approach was to examine the DNA sequences from GenBank website (www.ncbi.nlm.nih.gov/nucleotide) and determine which markers can be used as a species identification tool. We have designed new primers, tested in single as well as multiplex amplication of the four malaria species. Our results showed the multiplex PCR method is able to identify any of the five malaria species. Finally we compared our method with Singh's method and the traditional microscopic method to analyse over 100 blood samples. Our comparison indicated our multiplex method is faster, cheaper and as accurate.