DEVELOPING A MULTIPLEX PCR-BASED DIAGNOSTIC TEST FOR MALARIA

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Malaria is an endemic disease in most African countries, and it has been implicated as one of the major causes of morbidity and mortality worldwide. This is partly due to resistance evolved among the *Plasmodium* species and the difficulty in controlling its vector (Female *Anopheles* mosquitoes). Compared to microscopy and RDT, traditional PCR is highly specific and sensitive. However, it only amplifies one gene locus at a time. Very few assays target multiple loci in the same reaction. Hence, this study aims to develop multiplex PCR assays to amplify various diagnostic sequences in *Plasmodium falciparum* and *Anopheles* species, to provide additional information regarding drug and insecticide susceptibility in one test. To date, a highly sensitive and specific multiplex PCR assay targeting the *18S*, *pfmdr1* and *pfcrt* genes of *Plasmodium falciparum* has been developed using a set of primer combinations amplifying 395bp, 559bp and 145bp regions of the genes, respectively. Diagnostic sensitivity of the nested multiplex PCR is higher for the *mdr1* gene as the minimum detection limit is 88.04 copies of the *P. falciparum* genome in a reaction. It is proposed that the multiplex PCR has the potential to be a cost-effective, time-reducing, highly specific and sensitive test for malaria diagnosis. Further *Plasmodium* and *Anopheles* gene targets are being incorporated into this assay, and it is planned to apply next-generation sequencing to multiplex PCR amplicons products, which will provide additional data for control and treatment.