

DEVELOPMENT AND CHARACTERIZATION OF A REAL-TIME PCR ASSAY FOR THE DIAGNOSIS OF *PLASMODIUM FALCIPARUM* AND *VIVAX* FROM HUMAN BLOOD

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Microscopic examination of Giemsa-stained thick and thin blood smears is the reference standard technique for malaria diagnosis. This technique is time and labor intensive, requiring a high level of operator skill before maximal sensitivity and specificity can be achieved. Particularly problematic is the detection of low-level parasitemia, since microscopy can easily miss or mischaracterize such infections due to operator or sampling error. Consequently, the World Health Organization has prioritized the development of alternative malaria rapid diagnostic assays. Although PCR based assays generally enjoy higher sensitivity than microscopy or antigen capture methods, their role has largely been relegated to research applications because of the labor-intensive nature of traditional PCR. We have developed a sensitive and specific nucleic acid amplification-based assay for *Plasmodium falciparum* (PF) and *vivax* (PV) species targeting the 18S ribosomal RNA gene using real-time quantitative PCR (RTQPCR). Microscopically confirmed cases of PF and PV were identified in patients presenting to a malaria clinic in Maesod, Thailand. Real-time PCR results were compared to concordant results obtained from two independent expert microscopists. After excluding potentially misdiagnosed samples, PF and PV sensitivities at all parasitemias were 98.33% (95% CI: 91.1%-100.0%) and 100% respectively. PF and PV specificities were 96.15% (95% CI 86.8%-99.5%) and 98.41% (95%CI 91.5%-100.0%), respectively. This study highlights some of the known difficulties of microscopy based malaria diagnostics. Specificity of RTQPCR PV was disproportionately lower (75%, 6/24 false positives) at parasitemias of 1-100/ μ l, resulting from RTQPCR PV assay positives on microscopically determined PF samples. However, subsequent detection of an unrelated PV specific gene in many of these samples suggests that microscopy may have incorrectly diagnosed PF in patients actually harboring PV infection. The PF RTQPCR assay demonstrated no cross reactivity with the lone sample of *Plasmodium malariae* (PM) included in the study. Specificity was 100% (i.e. no false positives) in a pilot study using blood collected from malaria-naive subjects (USA), further suggesting that the microscopy/RTQPCR discordances observed at low parasitemias in patients from malaria endemic areas reflect true infection.

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