

Determining the prevalence and density of asymptomatic *Plasmodium falciparum* infections in low and high transmission settings in Western Kenya

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Background/Objective: Malaria remains a global health burden as 2018 saw 228 million cases worldwide. Pre-elimination efforts aim to decrease disease transmission, which depends on development of asexual parasites to sexual gametocytes within the human host. To better determine the relationship between asexual parasites and gametocytes, we aim to quantify both stages within the same blood sample by developing a multiplex RT-qPCR assay. We hypothesize that gametocytemia decreases linearly with asexual parasitemia at higher parasitemias but increases at lower parasitemia to improve transmission probability.

Methods: We conducted a cross-sectional survey of healthy participants aged 0 to 92 years at four sites in Western Kenya (Ajigo, Webuye, Kipsamoite, Kapsisywa) with varying levels of transmission to determine prevalence of asymptomatic *P. falciparum* blood-stage infections. Parasite density was quantified by light microscopy and by quantitative PCR of genomic DNA isolated from dried blood spots using *varATS* primers. For *varATS*-positive samples, we isolated RNA from whole-blood and performed stage-specific quantification by multiplex Taqman RT-qPCR targeting *PfPIESP2* (asexual), *Pfs25* (female), and *Pfs230p* (male).

Results: Among all participants, 127 of 1355 (9.37%) were positive for asymptomatic *P. falciparum* infection by *varATS*-qPCR. Using *varATS* positivity as the standard, multiplex RT-qPCR targeting *PfPIESP2* yielded a sensitivity of 78%. Across sites, overall parasite prevalence and gametocyte prevalence were highest in Ajigo (42.1%; 36%) followed by Webuye (10.5%; 9%) and Kipsamoite/Kapsisywa (0.873%; 0.1%). *Pfs25*-quantified gametocytemia decreased with decreasing *varATS*-quantified asexual parasitemia; however, the data poorly fit a linear model ($R^2 = 0.088$).

Conclusions: *PfPIESP2* detected 78% of *varATS*-positive samples, suggesting it may be poorly sensitive at detecting low-density blood-stage infections. Assay design may improve with a more sensitive gene target and further optimization. Based on quantification results, the

relationship between gametocytemia and asexual parasitemia appears direct but non-linear. Further work is necessary to better model the relationship between stage-specific parasitemia levels.