Detecting gametocytes: how sensitive is sensible?

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Improving our understanding of the human infectious reservoir of malaria parasites could expedite elimination of the disease. The presence of infectious *Plasmodium* gametocytes is requisite for onward transmission. The detectability of gametocytes depends on their density, the volume of blood examined, and methodology used. In a recent report, Essuman et al. identify a previously uncharacterised *Plasmodium falciparum* gene transcript, here named Pfg17 (PF3D7_1319800), for sensitive gametocyte detection. (1). In line with previous reports, Essuman and colleagues show that Pfg17 gene expression is strongly upregulated in gametocytes (2). On the basis of this transcription profile, the authors present a novel assay for gametocyte detection amplifying Pfg17 mRNA with a lower limit of detection than the most widely used assay amplifying Pfs25 (PF3D7_1031000) mRNA.

The strength of the study is the microarray analysis, which provides a short list of mRNA transcripts that are highly upregulated or specific to gametocytes. Though the Pfg17 assay requires further validation using standard qRT-PCR assays, in the form of biological and technical repeats with serial dilutions of purified gametocytes, and asexual stages to demonstrate its stage specificity, the assay may be a useful addition to the arsenal of tools for gametocyte detection.

The new assay's ability to estimate gametocyte density was not assessed in the current manuscript and we believe that is crucial for the assay to contribute to our understanding of *P. falciparum* transmission. As the authors point out, Pfs25's specificity to females limits its usefulness for gametocyte quantification when used in isolation. Male specific gene transcripts (PfMGET

[PF3D7_1469900], Pf230p [PF3D7_0208900]) enable the measurement of gametocyte sex ratio, which may be an important effector of gametocyte infectivity, and particularly informative during trials of gametocytocidal drugs that may have a differential effect on male and female gametocytes (3). Because Pfg17 appears more equally transcribed by male and female gametocytes (4) the assay may provide a uniquely accurate molecular estimate of total gametocyte density.

Recent studies indicate that gametocyte commitment is a stochastic process so, in all probability, some gametocytes form in the first rounds of erythrocytic schizogony (5). With progressive improvements in sensitivity, we may approach a stage where molecular tools simply bring gametocyte prevalence in line with total parasite prevalence. Sensitive detection of all malaria infections (without a specific focus on gametocytes) is, thus, argued to be of most relevance for public health intervention planning and evaluation (6). To better predict the infectious reservoir for malaria, accurate gametocyte quantification is more informative than sensitive gametocyte detection. For a mosquito to become infected after feeding, it must ingest a minimum of one gametocyte of each sex. With blood meal sizes anywhere between 2-8µL, both the Pfs25 and Pfg17 assays are capable of detecting gametocytes at concentrations below these minimal thresholds for infectivity (≥0.25 gametocytes/µL). In line with these theoretical limits, there is a clear decrease in mosquito infection rate at densities less than 1/µL (7). Our own studies show similar patterns; many individuals who are positive in Pfs25 based assays are not infectious to mosquitoes in membrane feeding assays, while the opposite is not observed.

A Pfg17 assay may form a highly desirable tool for characterizing the human infectious reservoir, provided it can robustly quantify male and female gametocytes across the entire range of densities that contribute to transmission. Importantly, future investigations should also examine the association of the measured gametocyte densities with the likelihood of mosquito infections.

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