

deep sequencing. Amplicon deep sequencing was performed using the highly-variable apical membrane antigen 1 (AMA1) as the target, a widely used *P. falciparum* genotyping marker. We validated the approach using mock mixtures of laboratory *P. falciparum* strains with the following characteristics: *i*) low starting DNA concentration commonly seen in low-parasitaemia infections (0.01 ng/mL = $\sim 3.5 \times 10^2$ genome copies/mL and 0.001 ng/mL = $\sim 3.5 \times 10$ genome copies/mL); *ii*) varying complexities of infection (three mock mixtures of MOI=1, 3 and 5) and *iii*) with uneven within-sample frequencies (MOI=1: 3D7 100%; MOI=3: FCR3 85.5% + DD2 10.5% + 3D7 4.0%; MOI=5: 7G8 50.0% + FCR3 25.0% + 3D7 15.0% + DD2 5.0% + HB3 5.0%). In addition, to test for the role of contaminating human DNA on protocol efficacy, three other mixtures were also made by spiking the MOI=3 *P. falciparum* mixture above with human DNA to obtain 10% parasite DNA and 90% human DNA. Finally, the protocol was then tested on clinical DNA samples extracted from *P. falciparum*-positive rapid diagnostic tests (RDTs) collected from Zanzibar, a low transmission setting where low density infections are common. Our findings suggest that a two-step protocol comprising sWGA and amplicon deep sequencing is a reliable and scalable technology which overcomes issues of low parasitaemia and low sample quality issues and allows accurate deep sequencing characterization of otherwise unsuitable field samples.

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GENETIC EVIDENCE OF CLONAL SPREAD, PARASITE EXCHANGE, AND UNIQUE SIGNATURES OF RELATEDNESS AMONG *PLASMODIUM FALCIPARUM* SAMPLES FROM HAITI

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Haiti is one of the last countries in the Caribbean with continued malaria transmission; thus, there is great interest in malaria elimination in this region. While much of the population of Haiti is at risk for malaria, there are a limited number of cases (17,622 in 2016), making conventional epidemiological measures such as case incidence and prevalence challenging. In this context, genetic signals are useful for the identification and characterization of the *Plasmodium falciparum* parasite population to identify *Foci* of transmission, detect outbreaks, and track parasite movement to inform elimination efforts. We evaluated 470 single-genome *P. falciparum* DNA samples extracted from dried blood spots from malaria-positive patients reporting to health facilities from primarily three Haitian departments (Nippes, Grand'Anse, and Sud). Initial genetic assessment using a 21-single-nucleotide polymorphism molecular barcode revealed evidence of clonal expansion within Nippes, in which 158/170 samples were an identical barcode type, as well as the divergence of a single clonal type in Grand'Anse. Furthermore, we detected the exchange of nine parasite lineages between Nippes, Sud, and Grand'Anse. In addition, 460/470 samples shared high levels of genetic similarity with at least one other sample in the dataset within 44 barcode-identical clusters. Based on this initial identification of a highly-related parasite population, we sought to further assess the relatedness of the parasites in these clusters. We performed whole-genome next-generation sequencing to characterize parasite relatedness as the percent of the genome shared between parasites as well as the length of stretches of genomes that were considered identical-by-descent. The results revealed patterns of relatedness suggestive of repeated recombination of a limited number

of founding parasite types without significant outcrossing. These genetic signals offer glimpses of the underlying relatedness of parasite populations and may be used to identify *Foci* of transmission against which targeted control efforts may be applied towards the elimination of malaria in Haiti.

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PROBABILISTIC CLASSIFICATION OF *PLASMODIUM VIVAX* RELAPSE AND REINFECTION USING MICROSATELLITE GENOTYPING DATA FOR IMPROVED ESTIMATION OF RADICAL CURATIVE EFFICACY

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Plasmodium vivax is a major cause of malaria outside Sub-Saharan Africa. Vivax malaria is characterized by repeated relapses which arise when formerly dormant liver hypnozoites are reactivated, leading to blood-stage infections. Relapse causes significant morbidity. The inability to distinguish relapse from reinfection hinders the assessment of antimalarial drug efficacy. Accurate estimation of radical curative efficacy therefore requires a probabilistic distinction of relapse from reinfection. We have developed a Bayesian framework for the probabilistic classification of recurrent vivax infections using polymorphic microsatellite markers. This method was applied to data from a randomized control trial comparing two primaquine radical cure regimens, and jointly models time-to-recurrence and *P. vivax* genetic data on nine microsatellite markers. A model of time-to-recurrence provides the prior probability. The genetic likelihood component integrates over all possible parasite relatedness networks within and across infections. We use a simplified model of identity by descent (IBD), whereby relapsing parasites can be clones (IBD of 1), meiotic siblings (IBD of 0.5), or strangers (IBD of 0), whereas parasites derived directly from reinfection are assumed to be strangers (IBD of 0). Preliminary results suggest that the efficacy of primaquine radical cure is greater than suggested from current standard clinical trial reports, with over 80% of recurrent infections following radical cure having a high probability of being reinfections rather than relapses. The day 7 carboxy-primaquine concentration (a slowly eliminated inactive metabolite) was a weak predictor of treatment failure (relapse). Probabilistic classification of relapse and reinfection significantly improves the estimation of the efficacy of radical curative regimens for vivax malaria. This method can be applied to radical cure efficacy trial data and, importantly, to other applications where the inability to distinguish relapse from reinfection presents a major challenge (e.g. to estimate incidence for the evaluation of intervention measures).

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POPULATION GENOMICS OF *VIVAX* MALARIA IN THE GREATER MEKONG SUBREGION

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Quick evolution and notable diversity in the genus *Plasmodium* has made effective drug, vaccine and diagnostic design a major hurdle to Malaria eradication. Further, it necessitates regional eradication programs tailored to specific Malaria populations. *Plasmodium vivax* (Pv) is the most common malarial pathogen outside of Africa, including in the Greater Mekong Subregion (GMS). Out of the 6 GMS countries, Myanmar has the highest documented Malaria burden, but very little is known about specific malaria populations in Myanmar, or the spread of Pv to and from nearby countries. In the past, population analyses for Pv in Myanmar and on its Chinese border have been limited by sample availability, among other factors. Using next generation sequencing data from Pv field isolates, we've addressed Pv variation around the China-Myanmar border both at