Development and application of highly multiplexed AmpliSeq targeted NGS assays for malaria genomic surveillance in Africa

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Emerging drug and diagnostic resistance highlight the urgency of continuous surveillance of malaria. Additionally, malaria vaccine rollout exerts unprecedented selection pressure on parasite populations. Genomic surveillance, which uses genetics to track diseases, aids in decision-making for malaria control and elimination. We developed a highly-multiplexed sequencing assay (AmpliSeq) for cost-effective targeted deep sequencing of *Plasmodium falciparum*. This assay integrates phenotypic and population genetic markers to study parasite dynamics in Sub-Saharan Africa and offers advantages over other methods through its large number of targets, full-length gene coverage, and exceptional sensitivity to low-density infections. The standardized, rapid protocol has been successfully piloted in Burkina Faso, the Democratic Republic of Congo, and Kenya. We demonstrate the application of the assay to investigate the prevalence and spread of markers associated with chloroquine, sulfadoxine-pyrimethamine and artemisinin resistance, and explore the rate of hrp2/3 deletions associated with false negative rapid diagnostic tests in West, Central and East Africa. In addition, we present the diversity of Pfcsp, the target included in malaria vaccines (both RTS,S and R21) and compared this diversity between continents, including also samples from Latin America and South East Asia. Our approach can effectively differentiate and characterize parasite isolates over time and space, and is easily adaptable to diverse epidemiological contexts. We show how results from these analyses can guide surveillance and implementation of control interventions such as chemotherapy and vaccine deployment strategies. The priority is to make this tool available to key actors in endemic countries to increase ownership and ensure data usage for decision-making and policy.

Eline Kattenberg: My career has focused on advancing malaria research through molecular epidemiology, surveillance tool development, and capacity building in endemic countries. During my PhD training in the Netherlands, I worked at the Royal Tropical Institute on malaria diagnosis and malaria in pregnancy, and after that as Section Head for Molecular Parasitology at the Papua New Guinea Institute of Medical Research, in joint appointment with the Walter and Eliza Hall Institute (Australia). Since 2016, I have been a Senior Scientist in the Malariology Unit at ITM Antwerp. My current work centers on malaria molecular epidemiology, population genetics, and the development of highly multiplexed targeted sequencing assays to strengthen molecular surveillance. These tools are now applied across Africa, Asia, and Latin America, and have provided new insights into drug resistance, population structure, and transmission dynamics of *Plasmodium falciparum* and *Plasmodium vivax*. I have prioritized capacity building, leading the molecular surveillance activities, incl. international training courses, supervising MSc and PhD students, and transferring molecular tools to partner laboratories in Vietnam, Peru, Burkina Faso, and the DRC. I aim to generate actionable data that guides targeted interventions, strengthens national surveillance systems, and accelerates elimination, built on equitable collaboration with endemic-country scientists whose contextual knowledge is indispensable.