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Commentary

"Resistance" to diagnostics: A serious biological challenge for malaria control and elimination

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Delay in diagnosis and treatment is the leading cause of death in malaria patients. The recommendation issued in 2010 by the World Health Organization (WHO) to reserve malaria treatment to parasitologically confirmed malaria infections has boosted the use of malaria rapid diagnostic tests (RDTs), which have now become a critical component of management and surveillance of malaria. Indeed, it has been estimated that over 280 million RDTs are now used annually, at a cost of hundreds of millions of euros [1]. Beyond their use as a diagnostic tool for patients with suspected malaria, the detection of *Plasmodium* antigens in blood samples is also used in *in vitro* tests of sensitivity to antimalarial drugs, as a marker of clinical severity and to verify the elimination of the parasite after treatment, although the decay of parasite antigens may take longer than the clearance of parasitaemia [2,3].

The vast majority of RDTs manufactured, purchased and used around the world are based on the detection of *P. falciparum* histidine-rich protein 2 (PfHRP2), alone or in combination with other antigens (*Plasmodium* lactate dehydrogenase [pLDH] and *Plasmodium* aldolase [pAldo]). PfHRP2 is a parasite-specific protein produced only by *P. falciparum* (and not the other human malaria species) throughout its asexual life cycle, and released during schizogony into the peripheral circulation, where it can persist for weeks after the elimination of parasites [3]. In 2010, a study sponsored by the World Health Organization (WHO) and the Foundation for Innovative New Diagnostics (FIND) established that some isolates of *P. falciparum* in Peru lacked the *pfhrp2* gene [4]. The *pfhrp3* gene is highly homologous to *pfhrp2*, and parasites lacking both *pfhrp2* and *pfhrp3* genes, or substantial parts of these genes, do not express functional proteins and are therefore not detected by PfHRP2-based RDTs. Such false negative results pose a life-threatening threat to case management, as patients really infected with *P. falciparum* may falsely be assumed to be malaria-free, and thus not managed adequately. They may also affect the efficacy of certain elimination strategies based on the RDT-based detection of malaria infections, such as reactive focal mass drug administration. Recently, numerous studies have reported *P. falciparum* parasites lacking *pfhrp2* and *pfhrp3* genes in Africa [5], with HRP2 deletion having been identified by WHO as one of the biological challenges currently threatening malaria control and elimination efforts. A mathematical model identified that a low intensity of transmission and a high frequency of treatment based on RDT detection of infection are the two main risk factors for the development of deletions [6]. Current WHO recommendations suggest the switch to non-PfHRP2 RDTs when the prevalence of pfhrp2-deleted parasites reaches the lower 90% confidence interval for 5% prevalence, or a plan for change if deletions are below 5% [7]. The high costs and resources required for this switch require quality data to avoid exhausting malaria control programs, particularly in the context of the generalized worse performance of non HRP2-based RDTs. Improved tools are also needed for surveillance at the local level and to estimate the expansion of parasites with genetic deletions. In addition, the investigation of the cause(s) of false negative RDTs in clinical settings is recommended, so as to identify the factors that may predispose to their emergence and expansion.

The approaches used for investigation, confirmation and reporting of *pfhrp2/3* deleted parasites have varied between studies and across countries [5]. The standard approach is a negative PCR result for the *hrp2* and/or *hrp3* gene on an agarose gel, complemented by additional PCR to confirm the presence of parasite DNA in the sample. However, new approaches based on quantitative PCR (qPCR) have been recently developed [8]. Moreover, immunoassays that detect and quantify HRP2 using bead-based multiplex assays allow the simultaneous detection of parasite aldolase, parasite lactate dehydrogenase and histidine rich protein 2 increasing the possibility of detecting gene-deleted parasites [9]. In an article in *EBioMedicine*, Kreidenweiss et al. [10] describe a novel probe-based, quantitative, real time PCR (4plex qPCR) that concomitantly confirms *P. falciparum* infections at submicroscopic levels, assesses *hrp2* and *hrp3* status and controls for DNA amplifiability in a

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single run. This highly sensitive method is a promising approach for cost-efficient population screening of *pfhrp2/3* deletions of large samples sets. Moreover, authors tested the assay in a cross-sectional, diagnostic accuracy study performed in Gabon, where they identified for the first time two *hrp2* negative *P. falciparum* parasites (2%).

These approaches still have some limitations. Low densities in some of the infections, especially among asymptomatic individuals, may affect the sensitivity of the assays. Moreover, the true prevalence of *P. falciparum* parasites with *pfhrp2/3* deletions will be affected by mixed infections with wild-type and mutant parasites, since these infections would still produce PfHRP2. Future work is needed to harmonize robust methods to prevent unnecessary recommendations for costly switches of RDTs in Africa. It is also needed to assess how much these deletions can affect surveillance estimates, their contribution in causing severe disease and deaths as well as their sensitivity to antimalarial drugs compared to wild-type parasites. However, important challenges remain to generate real-time data on the extent of this deletions with actionable potential for malaria control programs. The development of sensitive RDTs targeting parasite molecules other than HRP2 remains a priority for the correct management of malaria.

Declaration of Competing Interest

None.

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