P. falciparum hrp2/3 gene deletions

Conclusions and recommendations of a Technical Consultation
Geneva, Switzerland, 7-8 July 2016

Summary of conclusions and recommendations

Rapid diagnostic tests (RDTs) are a critical tool for malaria diagnosis in most endemic areas. The most common RDT target for the detection of Plasmodium falciparum is the antigen histidine-rich protein 2 (HRP2). The vast majority of RDTs manufactured, procured and used around the world are designed to detect HRP2 alone or in combination with other antigen/s. Monoclonal antibodies in RDTs target epitopes that are abundant in HRP2, as well as in HRP3, a structurally similar parasite protein.

Although the functions of HRP2 and HRP3 remain undefined, it is clear that they are not essential for parasite growth and transmission. Indeed, parasites can delete the genes encoding these proteins and continue to transmit in communities. Parasites with such gene deletions can cause false-negative results when HRP2-detecting RDTs are used. The first published clinical reports of infections with confirmed P. falciparum hrp2/3 gene-deleted parasites came from Peru in 2010. Additional reports from neighbouring countries in South America led WHO to recommend alternative diagnostic methods for affected areas; however, in recent years, pfhrp2/3 gene deletions have emerged in multiple endemic countries in Africa and Asia, causing concerns over malaria case management and control.

Although non-HRP2-based RDTs are commercially available, a rapid shift away from HRP2-based tests could pose serious supply security issues and disrupt the marketplace. In addition, there are few non-HRP2-based RDT options, as alternative detection systems are frequently less sensitive and are frequently more susceptible to heat and humidity.

In May 2016, the WHO Global Malaria Programme (GMP) published an information note for manufacturers, procurers and users of HRP2-based RDTs with interim guidance on how to investigate suspected false-negative RDT results, including those resulting from pfhrp2 gene deletions, and on alternative non-HRP2-based RDT options (http://www.who.int/malaria/publications/atoz/information-note-hrp2-based-rdt/en/). In parallel, a technical consultation on P. falciparum hrp2/3 gene deletions was held in Geneva on 7–8 July 2016. The consultation set out seven objectives. The final conclusions and recommendations related to these meeting objectives are proposed in bold text for the Malaria Policy Advisory Committee’s consideration.

1. Review the currently available data, and define the scope and scale of pfhrp2/3-deleted parasite populations based on published or in-press reports and recent unpublished investigations.

In 2014–2015, published reports of pfhrp2/3 deletions came from several countries in South America (Colombia, Brazil, Suriname and Bolivia), the China-Myanmar border and Ghana. Furthermore, unpublished data from studies investigating pfhrp2/3 gene deletions in Eritrea, India, Mozambique, Democratic Republic of Congo, Western Kenya, Western Indonesia, Uganda and Tanzania were reviewed.
during the meeting. In addition, preliminary results were reviewed from the Pf3k project, which is analysing the whole genome sequence of approximately 3000 geographically diverse \textit{P. falciparum} isolates for \textit{pfhrp2/3} deletions. Studies have varied in size; their inclusion of symptomatic versus asymptomatic patients; their use of prospective versus retrospective design; the availability of paired RDT and microscopy and/or PCR results; and the prevalence of single versus double \textit{pfhrp2/3} deletions (from 0\% in Mozambique and Western Kenya to 80\% double deletions in Eritrea).

Based on the data reviewed, it can be concluded that \textit{P. falciparum} parasites populations lacking one or both of the \textit{pfhrp2/3} genes are now present outside of South America in both high and low transmission areas and with varying prevalence across narrow geographic ranges. In South America, deletions were observed in parasite samples collected before HRP2-based RDTs were introduced and have spread with human migration; however, there is no strong evidence for the selection of \textit{pfhrp2/3}-deleted genetic alleles. Nevertheless, strong selection for \textit{pfhrp2}-deleted parasites may occur in areas where RDTs are used as the predominant diagnostic tool. A stochastic simulation model has found that theoretically, the use of HRP2-detecting only RDTs for the diagnosis and treatment of \textit{P. falciparum} malaria is sufficient to select for \textit{pfhrp2/3} double-deleted parasites. Given the public health implications of the continued use of HRP2-based RDTs where \textit{pfhrp2/3} deletions occur, WHO should promote a harmonized approach to investigating, surveying and reporting \textit{pfhrp2/3} gene deletions through the provision of standard protocols (including sample size calculations) and operating procedures. Furthermore, WHO should provide a list of reference laboratories that can provide full or partial support for PCR required to confirm (or exclude) \textit{pfhrp2/3} gene deletions, as well as laboratories that can perform complementary serological assays and targeted or whole genome sequencing. A harmonized approach will accelerate learning and future policy development.

2. Discuss options for the expanded mapping of \textit{pfhrp2/3} deletions

Generally, \textit{pfhrp2/3} surveys and surveillance activities should first target countries where deletions or concerns have been identified and in the neighbouring countries. Once deletions have been confirmed above a defined threshold (see below), continued surveillance and detailed mapping is not likely to be required because the action to be taken is dichotomous: to use or not use HRP2-only RDTs in the country.

Deletions identified and confirmed by a reference laboratory, referred through a range of scenarios, i.e., via complaint-reporting of suspected false-negative RDT results, or the retrospective analysis of discordant samples (HRP2-RDT negative and microscopy/PCR positive) from population surveys or small exploratory studies, should trigger prospective investigations such as i) community-based surveys around the index case(s); ii) geographically targeted hospital/health centre surveys of malaria suspects; iii) nationwide sentinel site surveillance of malaria suspects. All approaches should target symptomatic patients in all transmission settings; screen with either two RDTs (HRP2-based and non-HRP2-based, recommended by WHO) or an HRP2-based RDT and quality-assured microscopy. Blood for PCR confirmation of \textit{P. falciparum} infection and \textit{pfhrp2/3} gene analysis should be collected only from patients with discordant results (i.e., HRP2-RDT negative/non-HRP2-based RDT positive or \textit{P. falciparum} microscopy positive).

Where deletions have not been reported locally or in neighbouring countries, and when there is no evidence\textsuperscript{1} to suggest they are present, new initiatives to identify these gene deletions should not be prioritized. However, it is recommended that complaint-reporting mechanisms be strengthened and supervisors and NMCP staff be educated about \textit{pfhrp2/3} gene deletions. If resources are available, the recommended approach to screen for \textit{pfhrp2} gene deletions is to establish periodic sentinel site

\textsuperscript{1}Rates of discordance between RDT and microscopy results are systematically ≥ 10–15\%, with higher positivity rates with microscopy, where routine quality control is done by crosschecking or both are performed on the same individuals (e.g., during surveys); multiple formal complaints or anecdotal evidence of RDTs returning false negative results for \textit{P. falciparum}.
surveillance of symptomatic patients in all transmission areas where possible building on existing sentinel sites (e.g., for drug efficacy monitoring).

WHO should integrate *pfhrp2/3* gene deletions into the global mapping database currently under development.

3. Review and update current recommended procedures for investigating and reporting *pfhrp2/3* gene deletions

The published recommended procedures for investigating and accurately reporting *pfhrp2/3* deletions consist of three steps: 

**establishing initial evidence, confirmatory evidence, and prevalence** (Cheng Q et al., *Malaria Journal* 2014 13:283). Procedures should be **revised to indicate the roles and responsibilities of stakeholders at each level of the health system**, i.e., end-users, supervisors, national malaria control programme managers/MOH, reference laboratories, WHO.

In establishing the initial evidence, it was agreed that, given current workloads and capacities, front-line health workers can be asked to report, but not to investigate their own suspected false-negative RDTs. Health workers should report suspicious test results to their supervisors as part of routine reporting; if an explanation is not found, the supervisors should report the results to the NMCP. It is the NMCP that coordinates the investigation and subsequent response that generates the **initial and confirmatory evidence**. The national health authorities should **avoid** promulgating a message that all RDT-negative results are suspicious and/or that RDT results need to be confirmed with microscopy.

It is recommended that **confirmatory evidence include PCR for pfhrp3**, in addition to PCR for *pfhrp2*, as HRP3 proteins can show cross-reactivity in HRP2-based RDTs; however, the analysis of flanking genes for *pfhrp2* (and *pfhrp3*) and the serological confirmation of the absent HRP2 antigen (by ELISA or a second brand of RDT) are optional.

4. Develop a plan for technical support for countries conducting investigations into suspected *pfhrp2/3* gene deletions

*Pfhrp2/3* gene deletions are challenging to confirm and represent an urgent public health threat. Failure to recognize *pfhrp2/3* deletions raises the risks of false-negative *P. falciparum* infections going untreated or mistreated; of increased malaria transmission (due to failure to diagnose and treat infections); and of increased malaria morbidity and mortality. Unsubstantiated reports risk decreasing confidence in RDTs, and triggering unnecessary and costly changes to diagnostic strategy. Therefore, in order to promptly and effectively respond to this threat, **WHO should establish a consortium** made up of RDT procurers, NMCPs and their implementing partners, surveillance experts, malaria reference laboratories and research institutes to **provide technical support for the investigation of suspected false-negative RDTs due to *pfhrp2/3* deletions**, to establish appropriate surveillance systems, and to elaborate the factors influencing the emergence and spread of *pfhrp2/3* deletions.

5. Propose alternative RDT-procurement and case-management strategies for areas affected by *pfhrp2-deleted parasites*.  

A nationwide change to an RDT that detects non-HRP2 target antigens for *P. falciparum* is recommended when a prevalence threshold of patients carrying *pfhrp2-deleted parasites* meets or exceeds the lower 90% confidence interval for 5% prevalence\(^2\). If the prevalence is <5%, the recommendation is to plan for change over a longer time frame, as it is anticipated that *pfhrp2/3*-deleted parasites will persist and spread. Acquiring additional surveillance data would help to prioritize the roll-out of non-HRP2-based RDTs.

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\(^2\) This is to allow for sampling variability
In all other cases, if pfhrp2 deletions are confirmed in samples from any source, the suggested action is to establish prevalence through sentinel site surveillance or surveys.

A threshold of 5% was selected because it is around this point that the public health impact and proportion of cases missed by less sensitive non-HRP2-based tests is likely to be less than that associated with the continued use of HRP2-based tests.

Currently, the choice of non-HRP2-based RDTs that meet WHO’s recommended procurement criteria or WHO Prequalification requirements is very limited due to the reduced sensitivity and heat stability of such tests compared to HRP2-based RDTs. Tests with both HRP2 and pLDH antibodies on the same test line should be prioritized for assessment by WHO prequalification; assessment should include a laboratory evaluation against pfhrp2/3 single- and double-deleted parasites (culture and clinical samples) to determine whether they meet the recommended performance criteria. Programmes should not replace Pf-only HRP2-based RDTs with current HRP2/pan-pLDH or aldolase combination tests for the purpose of detecting non-HRP2-expressing parasites; only RDTs that specifically target pf-pLDH or pan-pLDH-only tests should be used.

6. Review the landscape of new tools for non-HRP2-based malaria diagnosis

Options for improving current pLDH-based RDTs exist, e.g., electronic readers, larger sample volume and related flow modifications; in addition, new nanoparticles, enzymatic labels, new or improved antibodies, and alternatives to HRP2-based RDT parasite detection are in development, e.g., cassette-based PCR, as well as field-adapted thermostable hydrogel PCR.

WHO should promote new test development and the improvement of existing tests, as well as the improvement of manufacturing processes. Furthermore, WHO should work with procurers to ensure an adequate pricing structure that will enable quality manufacture, and endeavour to accelerate prequalification/regulatory processes and field evaluations of new tests and electronic readers for non-HRP2-based malaria diagnosis.

7. Update WHO interim guidance on pfhrp2 gene deletions

The WHO interim guidance on investigating suspected false-negative RDT results and the implications of new reports of P. falciparum hrp2/3 gene deletions should be revised to reflect the conclusions/recommendations of the technical consultation and MPAC recommendations.