Background

Since May 2016, the WHO Global Malaria Programme (GMP) has published and updated an information note for the manufacturers, procurers and users of HRP2-based RDTs with interim guidance on how to investigate suspected false-negative RDT results, including pfhrp2.3 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 final conclusions and recommendations from this consultation were presented to the Malaria Policy Advisory Committee (MPAC) in September 2016 (http://www.who.int/malaria/mpac/mpac-sept2016-hrp2-consultation-short-report-session7.pdf).

This briefing paper provides an update on the current situation and planned next steps, based on the recommendations that emerged from the technical consultation and those received by MPAC at its last meeting (http://www.who.int/malaria/publications/atoz/mpac-september2016-report.pdf).

i) WHO should promote a harmonized approach to investigating, surveying and reporting pfhrp2/3 gene deletions through the provision of standard protocols (including sample size calculations) and operating procedures.

A standard protocol for estimating the prevalence of pfhrp2/3-deleted parasites (at province level) across geographical areas is in the final stages of revision and expected to be available in April 2017. The protocol will guide surveys estimating the prevalence of pfhrp2/3 gene deletions among symptomatic patients presenting to health facilities. This protocol will include a list of international reference laboratories and their respective capacities to support investigations. In addition, a set of external quality assessment (EQA) materials including pfhrp2/3 gene deletions will be prepared to support proficiency testing at these reference laboratories. Such materials will ensure reliability and comparability between laboratories. Run controls will also be made available for PCR quality control (see also point vi, below).

ii) Generally, pfhrp2/3 surveys and surveillance activities should first target countries where deletions or concerns have been identified, and the neighbouring countries.

WHO/GMP is actively supporting the design and planning of surveys for pfhrp2/3 gene deletions in the states/provinces of Ethiopia and Sudan bordering Eritrea. The surveys are expected to take place during the high-transmission season (September/October 2017). Sampling will be powered in order to obtain precise estimates of pfhrp2/3 gene deletions at the province level above or below the 5% threshold.
iii) WHO should integrate information about pfhrp2/3 gene deletions into the global mapping database currently under development.

The online global mapping database for insecticide and drug resistance has been adapted to accommodate reporting of pfhrp2/3 gene deletions (both positive and negative findings). A review was conducted of all published (and some unpublished) reports of pfhrp2/3 gene deletions, and data were extracted to inform the online global mapping database. The review yielded data from 20 countries and 110 distinct datasets.

Since the last MPAC meeting, new reports of pfhrp2/3 gene deletions have emerged from Rwanda, Uganda, Bangladesh and Mozambique.

iv) The published recommended procedures for investigating and accurately reporting pfhrp2/3 deletions are comprised of three steps: establishing initial evidence, establishing confirmatory evidence, and establishing prevalence (Cheng Q et al., Malaria Journal 2014 13:283). The methodology proposed in this paper should be revised to recommend 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 confirmation of absent HRP2 antigen (by ELISA or second brand of RDT) are optional.

The WHO information note has been updated to reflect these changes.

v) Pfhrp2/3 gene deletions pose an urgent public health threat, but they are challenging to confirm. Therefore, to promptly and effectively respond to the threat, WHO should establish a consortium to provide technical support in investigating suspected false-negative RDTs due to pfhrp2/3 deletions, to establish appropriate surveillance systems, and to elaborate on the factors influencing the emergence and spread of pfhrp2/3 deletions.

WHO/GMP has established a network of reference laboratories that will support investigations into suspected false-negative RDTs due to pfhrp2/3 gene deletions. More resources will be required to expand this network to include surveillance systems and the underlying environmental and biological factors driving the emergence and spread of pfhrp2/3 gene deletions.

As part of these harmonization efforts, during the ASTMH annual meeting, WHO/GMP co-organized a side-meeting on “Pfhrp2/3 gene deletions: Update, implications and response”. Reports of pfhrp2 gene deletions from various regions of the world were summarized, and models of RDT selection pressure and spread in Africa, policy implications and future directions were presented and discussed.

vi) Tests with both HRP2 and pLDH antibodies on the same test line should be prioritized for assessment by WHO prequalification, including a laboratory evaluation against pfhrp2/3 single- and double-deleted parasites (culture and clinical samples) to determine whether the tests meet 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.
WHO/GMP identified sources of culture-adapted isolates of *pfhrp2*-negative parasites in a small collection of archived wild-type HRP2-negative samples (200, 2000p/µl pairs) from Peru. With support from FIND, it initiated the prospective collection of *pfhrp2*-negative *P. falciparum* parasites in Peru (Universidad Peruana de Cayetano Heredia), which is now ongoing. These materials will be characterized and selected for inclusion in Round 8 of the WHO malaria RDT product testing panels, starting in March 2017. Round 8 is comprised of 35 RDTs and includes 10 products that target non-HRP2 antigens for detection of *P. falciparum*.

vii) Develop a plan of action for surveillance and response that can be supported by partners and implemented in countries.

An outline for a plan of action and response has been drafted, and an expert in infectious diseases and diagnostics who is familiar with the complexity and details of global malaria diagnostics, molecular biology, protein-ligand interactions, assay development, and HRP2 detection has been contracted to draft the document over the next 2 months. The contents are described below:

**Executive summary**

**Problem statement**

- History of RDT use and its current role in disease control and patient management (include types of tests, usage volumes and brief discussion of quality management mechanisms in place);

- Role of RDTs in achieving the goals of the *Global technical strategy 2016–2030*;

- Identification of HRP deletion mutants (history of our knowledge and evidence of the current size of the problem) [make point that not enough is known about the extent of the spread and clinical impact to take definitive action at present, but there must be a plan];

- Impact of RDT failure on tracking malaria incidence (versus other background diagnostic failures);

- Clinical and disease-control impact to date – examples from countries on how they are managing.

**State of knowledge and research gaps**

- Understanding the genetics of deletion (mechanism, genetic variability, drivers, fitness);

- Understanding the temporal origin of HRP deletion and mechanism of spread;

- Understanding technical factors that affect HRP2 detection (expression of HRP3, parasite density, circulating HRP2 antibodies, make of RDT and specific monoclonal used);

- Understanding the global epidemiology of deletions – the current size of the problem and the clinical impact;
• Modelling the future (with and without mitigation measures);
• Current status of pLDH-based RDTs and the hope for better RDTs (short- and medium-term): alternative falciparum markers that are abundant and species-specific.

Surveillance plan
• Clarifying the current size of the problem and its impact:
  o Strengthening laboratory networks (national and supranational) for surveillance and monitoring (standardized methods for sampling, testing, data management);
  o Options for mapping prevalence, i.e., which sites/countries/epidemiologic situations should be prioritized.
• Temporal and spatial tracking of HRP deletion prevalence:
  o Options for tracking over time (compare with other efforts in the longitudinal tracking of mutant types and mutation rates (e.g., AMR), lay out choices, costs and usefulness of different sampling methods);
  o Establishing triggers for action (prevalence cut-offs, outbreak sizes, trend speed).

Managing response
• Case detection and case management strategies at trigger points (dual testing, etc.);
• Risk communication with countries/national programmes;
• Engagement with the diagnostics industry;
• Procurers (cost constraints, complexity of procuring >1 RDT type and full product replacement);
• Changes required to WHO Product Testing;
• Interaction with regulatory/qualifying bodies.

Resource requirements to respond to the threat of \textit{pfhrp2}-deleted parasites

vii) Resource mobilization
The response plan will be utilized to mobilize resources to support the required actions. In the interim, WHO/GMP is investing a contribution from Bill & Melinda Gates Foundation (US$150 000) to support the following:

1. To develop a plan of action for surveillance and response to the emergence and spread of \textit{pfhrp2/3} gene deletions;
2. To develop a) standard protocols (and tools) for conducting baseline surveys and surveillance for the prevalence of \textit{pfhrp2/3} gene deletions, and b) report templates;
3. To establish a consortium of malaria reference laboratories and research institutes to provide methodologies and technical support and to conduct analyses for ruling in or excluding *pfhrp2/3* gene deletions. The outputs of the consortium will link with appropriate surveillance systems in order to map the emergence and spread of *pfhrp2/3* deletions;

4. To provide technical and financial support to countries investigating suspected *pfhrp2/3* gene deletions or implementing survey/surveillance activities. Priority will be given to countries at high risk due to their proximity to areas or countries with known high prevalence of *pfhrp2/3* gene deletions.