MESA FORUM

Updates on the WHO guidance for *pfhrp2/3* gene deletions surveillance and recently completed surveys

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Community of Practice *pfhrp2/3 gene deletions*
Mobilizing and providing peer and technical support

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Answers to pending Questions

Survey planning and implementation

1. **Is it possible to collect samples from asymptomatic people?**

   Collection of samples from asymptomatic people is not included in the WHO survey protocol templates because the focus is on assessing the impact of *pfhrp2/3* gene deletions on clinical malaria.
   If resources are available, asymptomatic persons with *P. falciparum* infection can be screened for *pfhrp2/3* gene deletions. There are examples in which *pfhrp2* gene deletions were identified in asymptomatic individuals but follow up studies amongst symptomatic cases presenting to health facilities did not reveal *pfhrp2* gene deletions. A possible explanation is lower virulence/fitness associated with *pfhrp2/3* gene deletions.

2. **The protocol we used in Kenya entailed a lot of paperwork (*i.e.* printing of several questionnaires). Would it be possible to incorporate alternative tools like ODK?**

   Yes, if NMCPs and/or research groups have the tablets or smartphones in sufficient quantity then an electronic version in an open-source platform could be developed.

3. **Are the recommendations in the guidance documents applicable also for Latin America?**

   Yes they are still applicable, however, several countries in Latin America decided many years ago based on surveys conducted at the time, that they would maintain reliance on microscopy and use RDTs – typically combined HRP2 and pfLDH and *P. vivax* – only in remote areas where microscopy is not feasible. Countries in this region should be considering a transition to RDTs with improved pfLDH based detection of *P. falciparum* – either alone or in combination with HRP2.

Molecular analyses

4. **What happens to countries that do not have accredited molecular reference laboratories?**

   WHO can connect you with the international reference laboratory experienced in these analyses. You may contact Jane Cunningham at: cunninghamj@who.int

5. **In the event of a mixed infection with 2 strains of Pf, one with a deletion and the other without deletion, would it not be possible to miss cases of deletion if molecular analyses are only done when we have a positive pLDH and a negative HRP2 test?**

   This is correct, mixed infections will not yield false negative results BUT they could be picked up by screening a subset of HRP2-positive and RDT negative samples using real-time PCR or digital PCR. Keep in mind that the survey protocol focuses on detecting the clinical impact of *pfhrp2/3* gene deletions and not on overall prevalence of these deletions – which needs to include symptomatics and asymptomatics, mixed infections.

6. **What could be the added value of AmpSeq in *pfhrp2/3* gene deletion detection?**

   Amplicon sequencing could allow for detection of minor clones in polyclonal infections that carry *pfhrp2/3* gene deletions (as can qPCR and droplet digital PCR methods). AmpSeq provides haplotype-level resolution, which can help track the spread and emergence of deletion-carrying strains; it can differentiate between independent deletion events and clonal expansion of a single deletion strain.

   Furthermore, AmpSeq panels can include other resistance markers (*e.g., pfcrt*, *pfmdr1*, *K13*, etc.) so it allows for simultaneous (and integrated) genotyping of additional markers, if there is good rationale. It can also serve as a confirmation of PCR results – particularly false positive *pfhrp2/3* gene deletions due to PCR failure.

   Another advantage is that AmpSeq datasets can be reanalyzed in the future as new information becomes available. However, despite these benefits, it is important to consider the higher cost and technical complexity compared to PCR alone, as it requires both bioinformatics capacity and robust data pipelines.

Plasmodium spp.

7. **If microscopy reveals that a participant is infected with mixed *Plasmodium* species, can the sample be used for *pfhrp2/3* genotyping? Or is it only for mono-infections?**

   Yes, mixed species samples can be used. In the genotyping methods targeting *P. falciparum*-specific sequences (*e.g.,* species-specific PCR primers or AmpSeq panels), non-falciparum DNA does not interfere, provided the primers have a good specificity.

8. **Slightly off-HRP2/3-topic, but in both surveys negative HRP2 RDTs were found to be due to non-Pf infections. We know from routine these cases are also found (when verifying negative HRP2 results by microscopy, where this is feasible), and it decreases trust in mRDT even when it's a very low frequency. Does WHO have any recommendation on a threshold of non-Pf prevalence for considering a combined RDT (HRP2/pan-pLDH) or a non-HRP2 RDT?**

No, WHO does not have specific recommendations about this. The value will very much depend on the epidemiology of the setting – for example if the majority of *P. malariae* infections are mixed with *P. falciparum* – then the benefit may be marginal. The performance of the pan-lines for selection of these infections is also likely to vary. We are planning to review literature on RDT performance for detection of *P. malariae* and *P. ovale* in the near future to help better articulate such guidance.

Antimalarial drug resistance and TES

9. Other than diagnostic issues, do these HRP2/3 deletions also have an effect on response to the drug treatment or emergence of artemisinin resistance?

No, *pfhrp2/3* genes/proteins are not involved in drug metabolism or drug targets, so treatment efficacy is unaffected by their absence.

Recent studies, particularly from Eritrea, have shown a higher prevalence of K13 mutations in *pfhrp2/3*-deleted parasites, raising important questions about potential links between diagnostic evasion and antimalarial drug resistance. These issues could be happening separately or possibly a single lineage is acquiring both survival advantages and expanding clonally. This may suggest that *pfhrp2/3*-deleted strains are persisting longer due to diagnostic evasion, giving them more time to accumulate and spread resistance mutations. This is an area for ongoing study.

10. In the current world situation with the restriction of the funding to the country, would it be possible to use samples from another study, such as TES, for surveillance of HRP2/3 deletions?

Yes, it is possible if the ethics approval and patient consent are compatible with this additional testing. Particular attention is needed to draw appropriate conclusions because the study may not have been appropriately powered or representative to inform RDT decision making.

11. When do we expect the update of the TES Protocol that includes concurrent detection of *pfhrp2/3* gene deletions?

Will be finalized by the end of this year.

RDT switching & country decision making

12. What should countries do with those RDTs in circulation that are no longer prequalified by WHO?

We suggest you transition to prequalified RDTs but you can use the stocks that are in place.

13. **In a scenario where only a small number of sites exceed the threshold while the majority remain below it across 18 states, how can this evidence effectively drive policy change? Given the challenges in implementing new policies, what type of policy adjustments would be most appropriate? Additionally, what strategies can be used to manage the transition period until the new policy is fully implemented?**

    WHO recommends changing to alternative RDTs NATIONALLY if there are any domains/regions over the 5% threshold. This is because we anticipate deletions will spread. Having data from many different areas of the country informs how to prioritize roll out of new RDTs. A realistic time frame for changing RDTs depends a lot on the size of the country and existing stocks.

14. **How affordable are these combined new tests compared to the current conventional RDTs?**

    There is no set price, but we know from experience of countries who have had to change RDTs (Eritrea, Djibouti) that the price is significantly higher. We hope with big volumes the price will be perhaps 10-15 cents more/test.

15. **What will be the fate of the deleted parasite populations, are they increasing or decreasing after switching RDT?**

    We do not have data on repeat surveys from many places over time. In Eritrea, although there were no direct comparisons, the overall prevalence of deletions appears to have decreased. However, in Peru, where RDT pressure did not play a significant role, the prevalence of deletions has increased over time. We anticipate more data emerging in the next couple of years.

16. **My question is about the future use of RDT. If HPR2-detecting RDTs are no longer recommended for case management diagnosis worldwide, will we depend on LDH detection based or we have another alternative in mind?**

    It is most likely that combination HRP2 and pfLDH RDTs will replace HRP2-only RDTs. The HRP2 and pfLDH may be on the same or separate lines. This is because despite significant improvements in performance of pfLDH test lines, overall performance is highest with combined antigen targets [ref].

Supporting resources

17. **Can you post the link of the *pfhrp2/3* Planner App?**

    The *pfhrp2/3* Planner App can be found here.

18. **Are there any relevant trainings or conferences to learn more on this topic?**

    There are no training workshops currently planned due to resource constraints but technical support is available through contact with WHO GMP and the network of reference laboratory focal points.