PfHRP2 deletions: an emerging threat?

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Malaria RDTs

- **Type of RDTs**
  - Pf only: PfHRP2 or PfHRP2/PfLDH
  - Pf/Pan: PfHRP2/pLDH, PfHRP2/aldolase
  - Pf/Pv: PfLDH/PvLDH
  - Pf/Pvom: PfHRP2/pLDH
  - Pf/Pf/Pv: PfHRP2/PfLDH/PvLDH
  - Pan only: pLDH

- **RDTs evaluated by the WHO-FIND RDT Evaluation Program**
  - 251 RDTs evaluated in Round 1-6
  - 65 RDTs met recommended WHO procurement criteria
  - 58/65 RDTs are HRP2 based
**pfhrp2 and pfhrp3**

- *P. falciparum* specific
- Functions unknown
- Not essential for parasite growth and transmission
### Pf isolates collected from Iquitos, Peru in 2007

<table>
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<tr>
<th>Isolates</th>
<th>LM</th>
<th>ICT</th>
<th>CareStart</th>
<th>Pf Combo</th>
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</table>

Gamboa et al PLoS One 2010
**Amplification of pfhrp2 and pfhrp3**

Gamboa et al. PLoS One 2010
Amplification of other genes

18s rRNA

+ -

pfmsp1

+ -

pfglurp

pfmsp2

Gamboa et al PLoS One 2010
Amplification of flanking genes

MAL7P1.230

MAL7P1.228

MAL13P1.485

MAL13P1.475

Gamboa et al PLoS One 2010
# Parasites lacking pfhrp2 and pfhrp3

| Isolates  | LM | ICT | CareStart | Multiplex | Allelic Type | MAL7 | hrp2- | MAL7 | MAL13 | hrp3- | hrp3- | MAL13 | ELISA | ELISA |
|-----------|----|-----|-----------|-----------|--------------|-----|------|-----|------|-------|-------|------|------|------|-------|
|           | P| rRNA | Pfmsp1/ | P1.230 | exon | exon 2 | P1.228 | P1.485 | exon | exon | exon 2 | P1.475 | HRP2 | pLDH |
| Combo     | pfmsp2/pfglurp | -5.535kb | 1-2 | +6.49kb | -4.404kb | 1-2 | 1-2 FL | +1.684kb | (ng/mL) | (ng/mL) |
| PE01F04*  | + | + | + | Pf | A/1/I | + | + | + | + | + | + | + | + | 235 | 503.4 |
| PE01F06   | + | + | - | Pf | A/1/I | - | - | - | + | + | + | + | + | 0 | 19.27# |
| PE01F07   | + | - | - | Pf | B/2/II | - | - | - | + | + | - | - | + | - | 0 | 3534.75 |
| PE01F011* | + | - | - | Pf | A/2/III | - | - | - | + | + | - | - | + | - | 0 | 698.25 |
| PE01F015  | + | + | - | Pf | A/2/IV | - | - | - | + | + | + | + | + | 0 | 41.85 |
| PE01F016* | + | - | - | Pf | A/2/IV | - | - | - | + | - | - | - | - | - | 0 | 38.65 |
| PE01F017  | + | - | - | Pf | A/2/IV | - | - | - | + | - | - | - | - | + | 0 | 467.1 |
| PE01F018* | + | - | - | Pf | A/2/IV | - | - | - | + | - | - | - | - | - | 0 | 117.7 |
| PE01F019* | + | - | - | Pf | A/2/IV | - | - | - | + | - | - | - | - | - | 0 | 61.3 |
Parasites with \textit{pfhrp2} and \textit{pfhrp3} deletions in Peru (2003-2007)

Gamboa et al PLoS One 2010
Non-HRP2 detecting RDTs or LM should be used in affected areas
Evolution of parasites lacking HRP2 in Peru

Figure 4 | Prevalence of *pfhrp2* among the clonal lineages identified in Iquitos. Clinical samples were collected in 1998–2001 (N = 92) and 2003–2005 (N = 96). Dark grey boxes represent the proportion of *pfhrp2*-positive samples while light grey boxes represent *pfhrp2*-negative isolates. The clonal lineage assignments are indicated along the x-axis.

Pfhrp2 deletions in most lineages

Akinyi et al 2013 Scientific Reports
Parasites Lacking HRP2

Proportion of P. falciparum

- No estimated malaria
- 0–20 Pf; 80–100 Pv
- 20–40 Pf; 60–80 Pv
- 40–60 Pf; 40–60 Pv
- 60–80 Pf; 20–40 Pv
- ≥80 Pf; ≤20 Pv

Gamboa et al 2010
Maltha et al 2012
Akinyi et al 2013
Houze et al 2011
Trouvay et al 2013
Murillo et al 2015
Abdallah et al 2015
Koita et al 2012
Ramutton et al 2012
Wurtz et al 2013
Amoah et al 2016
Laban et al 2015
Kumar et al 2013
Bharti et al 2016
Li et al 2015

Impact of reporting *pfhrp2* deletion

- **Under report**
  - False negative *P. falciparum* infections not/mistreated
  - Increasing transmission
  - Increasing morbidity and mortality

- **Accurate report**
  - Triggers changes of RDT products
  - Implements other diagnostic tests
  - Ensures appropriate case management

- **Unsubstantiated report**
  - Decreases confidence in RDTs
  - Triggers unnecessary costly changes of RDTs
Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting

Qin Cheng¹, Michelle L Gatton², John Barnwell³, Peter Chiodini⁴, James McCarthy⁵, David Bell⁶ and Jane Cunningham⁷*

Abstract

Malaria rapid diagnostic tests (RDTs) play a critical role in malaria case management, surveillance and case investigations. Test performance is largely determined by design and quality characteristics, such as detection sensitivity, specificity, and thermal stability. However, parasite characteristics such as variable or absent expression of antigens targeted by RDTs can also affect RDT performance. Plasmodium falciparum parasites lacking the PfHRP2 protein, the most common target antigen for detection of P. falciparum, have been reported in some regions. Therefore, accurately mapping the presence and prevalence of P. falciparum parasites lacking Pfhrp2 would be an important step so that RDTs targeting alternative antigens, or microscopy, can be preferentially selected for use in such regions. Herein the available evidence and molecular basis for identifying malaria parasites lacking PfHRP2 is reviewed, and a set of recommended procedures to apply for future investigations for parasites lacking PfHRP2, is proposed.
Recommended Procedures

Establish initial evidence
Establish confirmatory evidence
Establish prevalence
Step 1. Establish Initial Evidence

- LM positive for Pf (2 qualified microscopists)
- Parasitemia counted
- PfHRP2 band negative on two RDTs of a quality product
- PAN band positive if applicable
- Confirmed by PCR

HRP2-based RDTs fail to detect Pf infections with reasonable parasite densities, not due to poor LM or low density.
Step 2. Confirmatory Evidence

Gene deletion analysis

- PCR fails to amplify pfhrp2 exon 2 and/or exon 1-2
- PCR is able to amplify > 2 single copy genes of Pf
- PCR fails to amplify one or both flanking genes (optional)
- PCR fails to amplify pfhrp3 exon 2 and/or exon 1-2
- PCR fails to amplify one or both flanking genes (optional)
- Whole genome sequencing

pfhrp2 is deleted, not undetectable due to insufficient DNA

pfhrp3 is deleted
Step 2. Confirmatory Evidence

Antigen analysis (optional)
- PfHRP2 band negative on a 2nd brand of quality RDT
- PfHRP2 ELISA negative

PfHRP antigens are not produced
Step 3. Establish Prevalence

- Prospective surveys in countries where HRP2 deletion has been confirmed or in neighbouring countries
  - Community based survey around the index case(s)
  - Geographically targeted hospital/health centre survey of malaria suspects
  - Nationwide sentinel site surveillance of malaria suspects
- Target symptomatic patients
- Screen with either two RDTs (HRP2 based and non-HRP2 based, recommended by WHO) or a HRP2 based RDT and quality-assured microscopy.
Investigation of parasites lacking HRP2/3

- Health workers
  - Record results
  - Store RDT
  - Report suspected false negative RDT results

- Supervisors
  - Trouble shooting
  - Report suspected false negative RDT results

- NMCP
  - Establish initial evidence
  - Collect samples
  - Contact reference labs for confirmation

- Reference labs
  - Establish confirmatory evidence

Prevalence Surveillance
False-negative RDT results and implications of new reports of *P. falciparum* histidine-rich protein 2/3 gene deletions

MAY 2018

INFORMATION NOTE

TARGET AUDIENCE

National malaria control programmes (NMCP) managers and their implementing partners, procurement agencies, national regulatory authorities for in-vitro diagnostics and manufacturers of malaria rapid diagnostic tests (RDT).

PURPOSE

To provide information on the implications of recent reports of histidine-rich protein 2/3 (*pfhrp2/pfhrp3*) gene deletions in *P. falciparum* parasites for case management in Africa and to advise on procedures for investigating suspected false-negative RDT results.

BACKGROUND

Most of the currently available commercial RDT kits work by detecting a specific protein expressed only by *P. falciparum*, called HRP2, in the blood of people infected with *falciparum* malaria. The antibodies on the test strip recognize the HRP2 antigen but may cross-react with another member of the HRP gene family, pHRP3, due to strong similarity of the amino acid sequence. The general preference for HRP2-based RDTs in procurement is due largely to the finding in some studies that they are more sensitive and heat-stable than RDTs that detect other malaria antigens, such as plasmodium lactate dehydrogenase (pLDH) – pan (all species) or *P. falciparum*-specific – or aldolase.
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</table>
Acknowledgements

❖ Peru team
   Dionicia Gamboa, Jorge Bendezu, Katherine Torres
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