Pfhrp2 deletions and other diagnostic challenges

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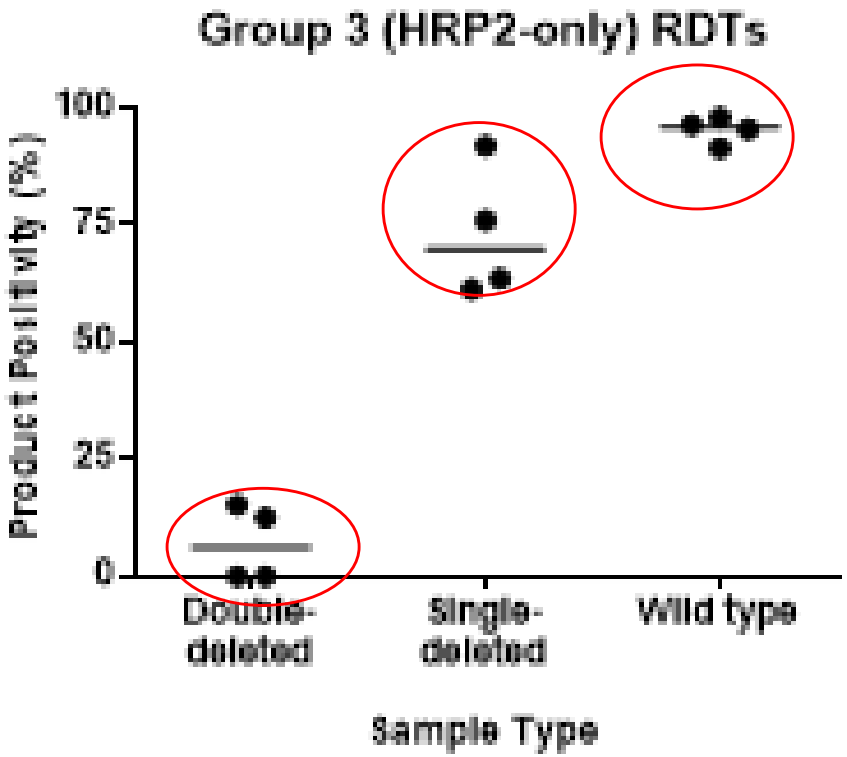
Global **Malaria** Programme

World Health Organization

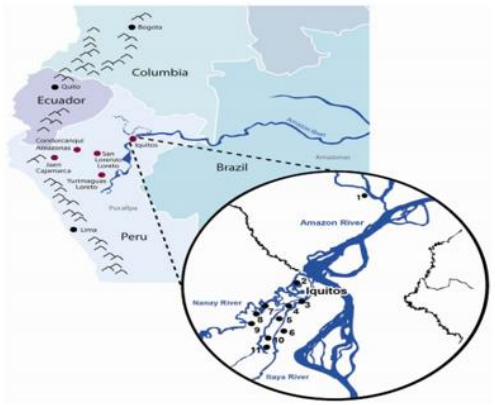
HRP2 – histidine rich protein -2

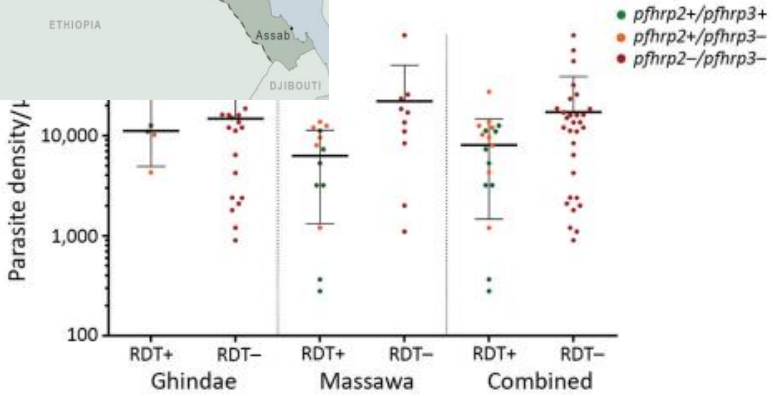
- HRP2 is found in the cytoplasm and surface of Pf-infected erythrocytes; it's produced in abundance but function is not very well understood (number of theories) and Pf parasites can survive without it
- HRP3 close cousin – share common epitopes
- RDTs that target HRP2 can to some extent also detect HRP3 (>1000p/µL, brand)
- This cross-reactivity is critical to reducing clinical impact of *pf-hrp2* deletions
- Knowledge of pfhrp2 deletions alone doesn't predict RDT performance

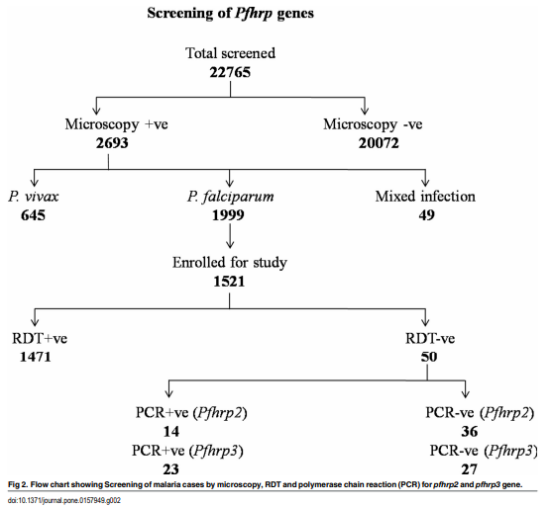


Source: Malaria RDT Test performance: WHO Product Testing Round 8 (2016-2018)

First reports in Peru in 2010 …Turning point in 2016







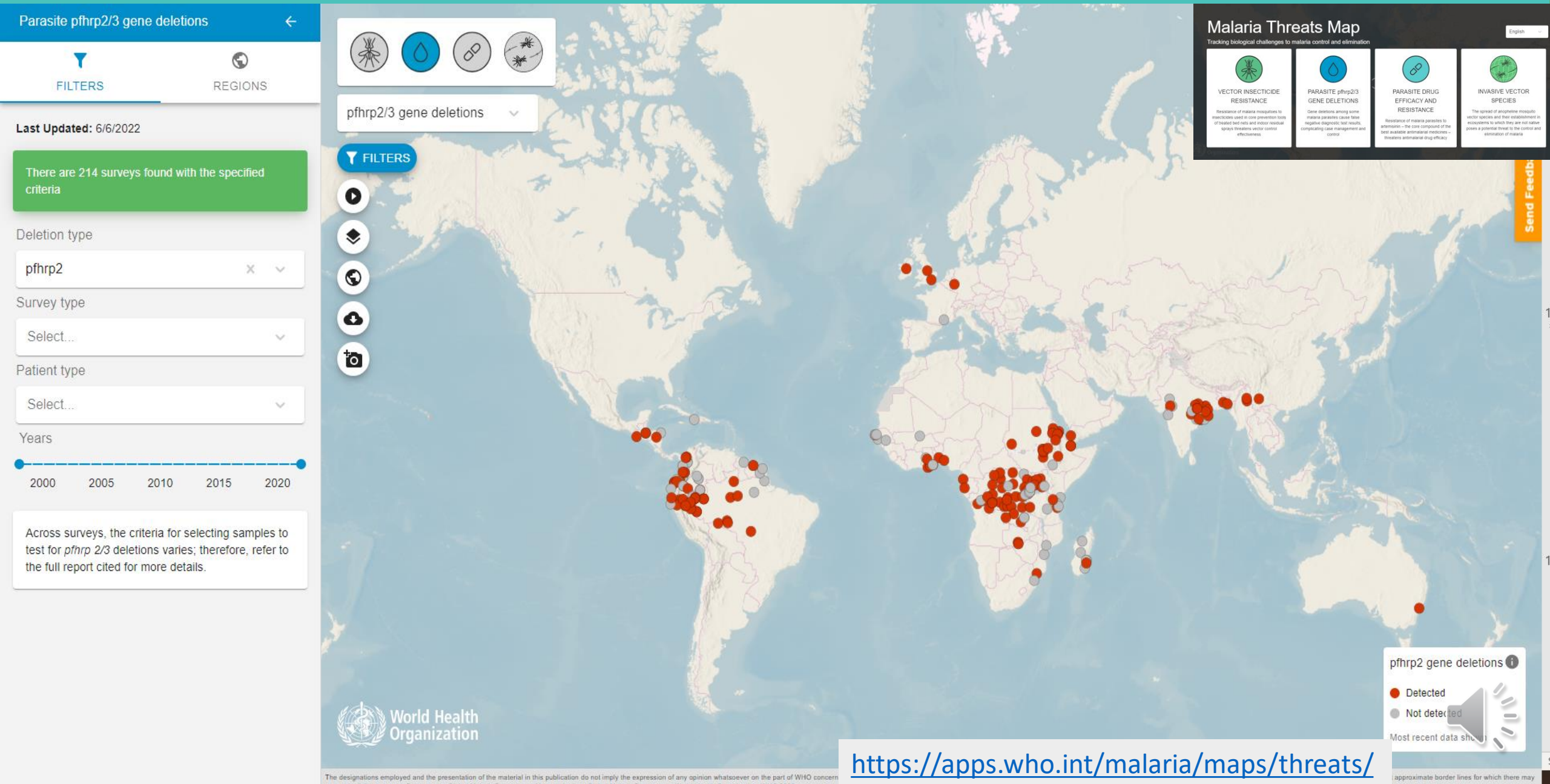
- 41% (61/148) of isolates lacked *pfhrp2;*
- 21% lacked both *pfhrp2 and 3*

Very high prevalence of double deletions in Eritrea and overall low but heterogeneous prevalence of deletions in India (eight states)

Berhane A, et al. Major Threat to Malaria Control Programs by Plasmodium falciparum Lacking Histidine-Rich Protein 2, Eritrea. Emerg Infect Dis. 2018 Mar;24(3):462-470.

Bharti PK et al (2016) Prevalence of pfhrp2 and/or pfhrp3 Gene Deletion in Plasmodium falciparum Population in Eight Highly Endemic States in India. PLoS ONE 11(8): e0157949.

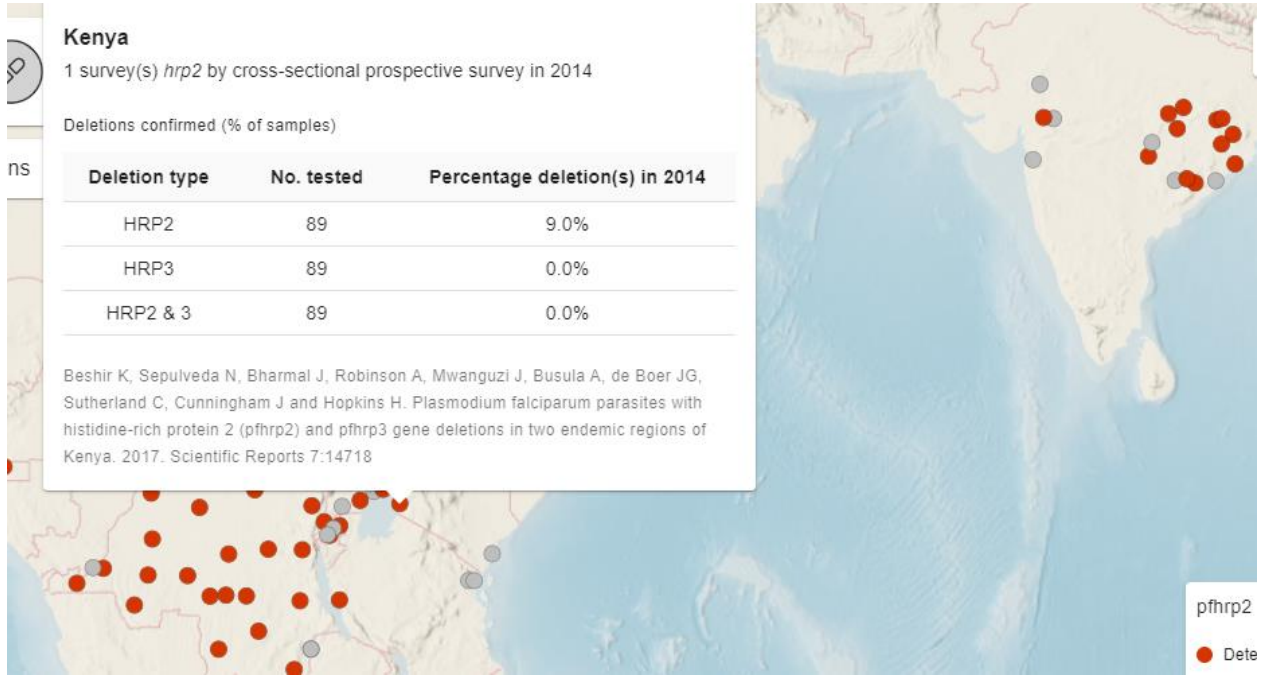
How do we track ? WHO Malaria Threat Maps



https://apps.who.int/malaria/maps/threats/

Getting at the true picture ….

- Malaria threat maps chart what is in the published report – typically percentage of pfhrp2 deleted samples amongst those tested and NOT all *P.falciparum* cases
- Populations are different – age, symptoms/no symptoms, selection criteria for genotyping
- RDT result not always known – don't know if the deletion led to a false negative result
- Original source is required to properly interpret the results.
- <u>CANNOT CURRENTLY USE MAP TO DETERMINE WHERE POLICY SHOULD CHANGE</u>



Way forward – complementary dashboard of planned and ongoing surveys; indicate where RDT policy has changed

When to suspect HRP2 deletions ?

- In a <u>patient</u>
  - negative results on an HRP2 test line of at least two quality-assured malaria RDTs

  And

  - positive on the pan- or pf-pLDH test line, when a combination test is used

  And

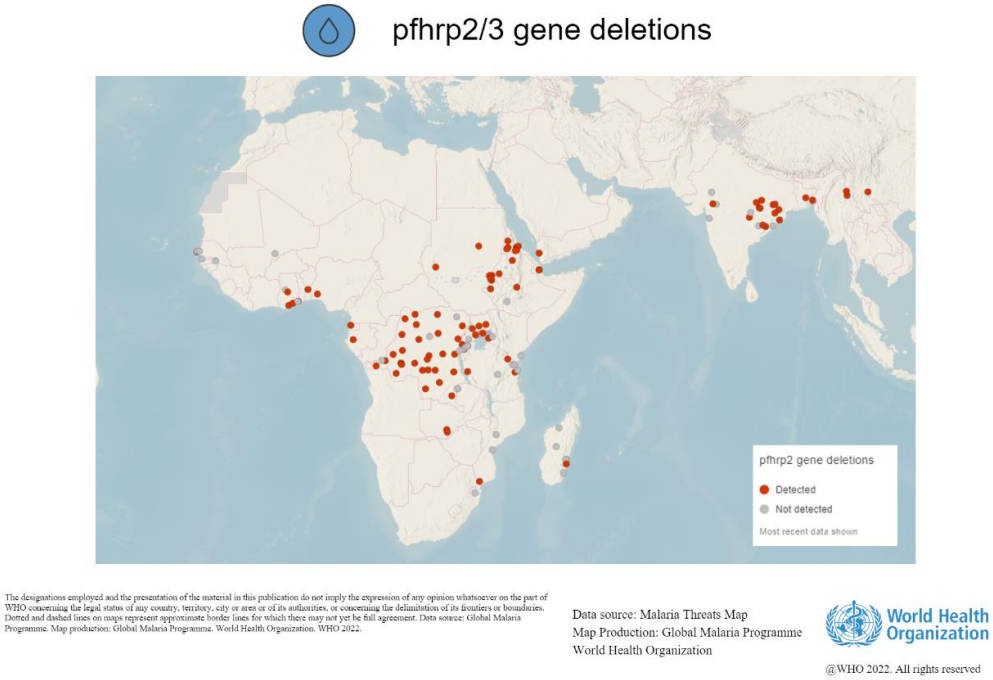
  - the sample is confirmed microscopically to be positive for *P. falciparum* by two qualified microscopists.

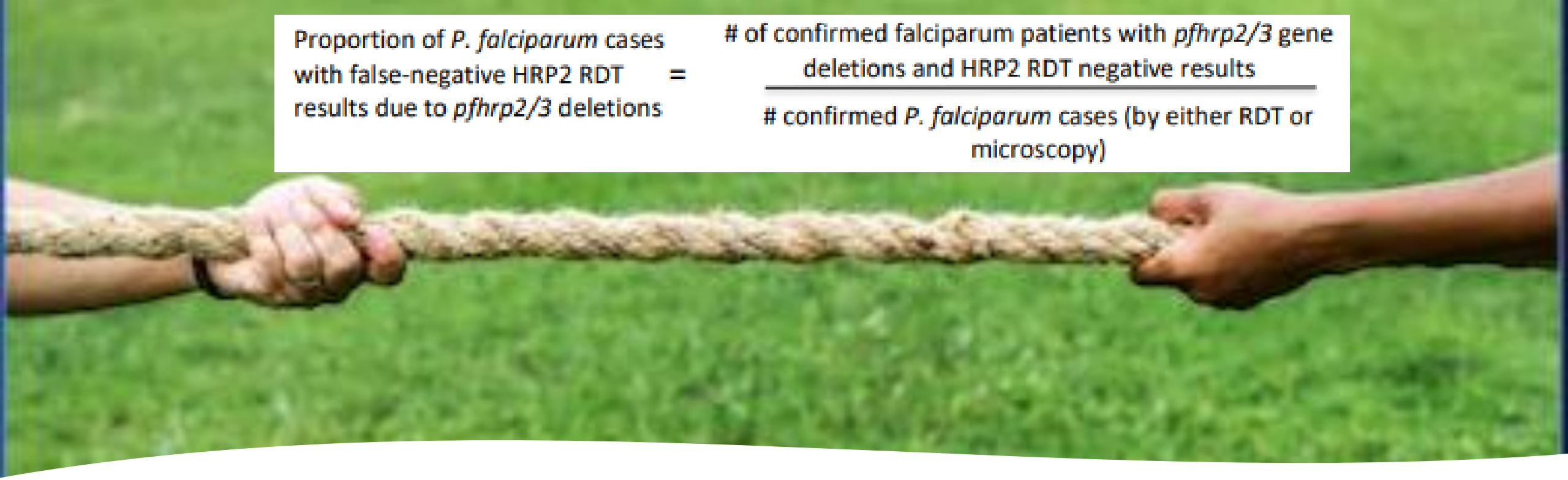
- Also consider travel history to areas with high prevalence of HRP2 deletions e.g. Peru, Brazil, Eritrea, Djibouti, Ethiopia

https://apps.who.int/iris/bitstream/handle/10665/258972/WHO-HTM-GMP-2017.18-eng.pdf?sequence=1

When should a programme be suspicious ?

- in a programme, the rates of discordance between the results of RDTs and microscopy are systematically ≥ 10–15%, with higher positivity rates in microscopy,
- when the national malaria control programme receives multiple formal complaints or anecdotal evidence of RDTs that give false-negative results for *P. falciparum.*
- When *pfhrp2/hrp3* gene deletions have been reported, the baseline prevalence should be determined in the affected country and neighbouring countries





Proportion of *P. falciparum* cases with false-negative HRP2 RDT results due to *pfhrp2/3* deletions = $\frac{\text{\# of confirmed falciparum patients with } pfhrp2/3 \text{ gene deletions and HRP2 RDT negative results}}{\text{\# confirmed } P. falciparum \text{ cases (by either RDT or microscopy)}}$

When to switch away from HRP2 based RDTs

- the prevalence of symptomatic patients carrying pfhrp2-deleted parasites causing false-negative HRP2 RDT results is ≥ 5%
- A threshold of 5% was selected because it somewhere around this point that the proportion of cases missed by HRP2 RDTs due to non-hrp2 expression may be greater than the proportion of cases that would be missed by less-sensitive pLDH-based RDTs
- Comparing sensitivity of HRP2-RDTs and pf-LDH RDTs to microscopy or PCR in several studies the difference is <5-7% amongst symptomatic individuals

What contributes most to missing cases ?

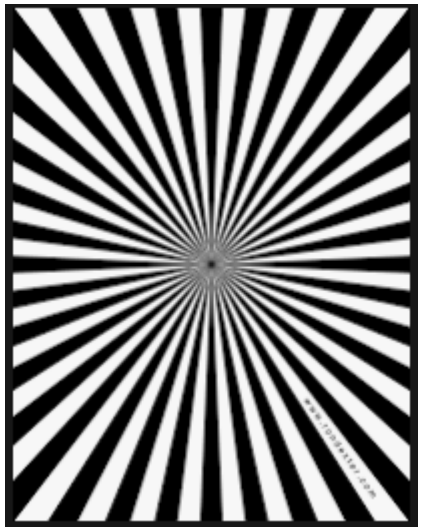
- HRP2-RDT negative due to pfhrp2/3 deletions

pf-LDH (or pan-LDH) RDT negative or faint line missed due to low density infection

Why might *pfhrp2* deletions not result in negative HRP2-RDTs?

- Multiclonal infection with wild-type and *pfhrp2* deleted *P.falciparum*
  - Possible to detect using mulitplex real time or digital drop PCR but not conventional PCR
- Residual HRP2 from previous Pf infection and current infection with deleted parasites
- Pfhrp3 is present and antibodies on the RDT strip react with common epitopes

Focus is on clinically relevant *pfhrp2/3* deletions
- screening symptomatic populations
- prioritizing molecular analysis of samples that have discordant RDT results : HRP2 negative and pf or pan-LDH positive



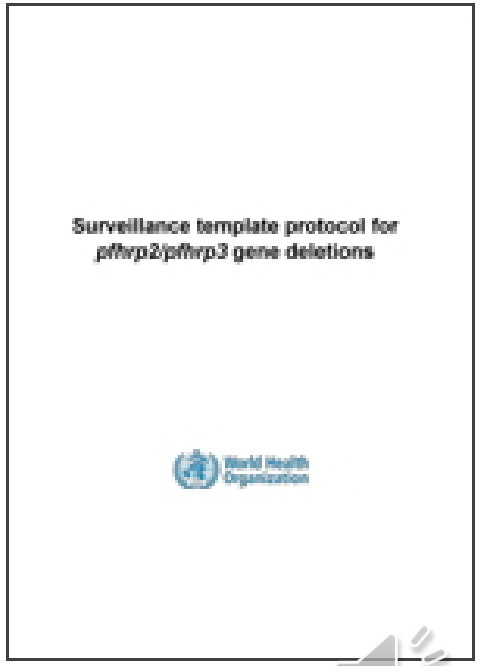
WE KNOW THIS APPROACH UNDERESTIMATES TRUE PREVALENCE OF PFHRP2/3 DELETIONS

- A recommendation to switch is further informed by mathematical models that show whether parasites lacking pfhrp2 genes will spread under HRP2-only RDT pressure; a switch may also be decided because of the complexity of procuring and training in use of multiple RDTs.
- **Any change should be applied nationwide, although roll-out might be prioritized on the basis of the prevalence of *pfhrp2* deletions.**

WHO Response Plan to *pfhrp2/3* deletions

Core response plan to pfhrp2/3 deletions

- **mapping the distribution and frequency of *pfhrp2/3* deletion mutants with harmonized protocols;**
- building an international network of laboratories to perform the complex molecular confirmation required for mapping and identify new and/or efficient screening methods ;
- supporting countries in the selection and procurement of new RDTs when a change of testing is warranted;
- advising commercial manufacturers of the priorities for new tests and providing the best available market forecasts;



https://apps.who.int/iris/bitstream/handle/10665/325528/WHO-CDS-GMP-2019.02-eng.pdf?sequence=1&isAllowed=y
https://apps.who.int/iris/rest/bitstreams/1270340/retrieve
https://apps.who.int/iris/bitstream/handle/10665/331197/9789240002050-eng.pdf
http://www.mesamalaria.org/resource-hub/resource-compilation-responding-threat-pfhrp23-deletions

What are the alternatives ?

- HRP2 RDTs most sensitive and heat stable
- Profit margins small therefore little new investment to improve non-HRP2 targets
- Only one WHO prequalified pan-LDH-only product -and that manufacturer has 'notice of concern'
- supply risk and no combo test (Pf-LDH, Pv-LDH) that meets WHO criteria!; Pf-pan-LDH alternatives lead to misclassification of Pf as non-Pf – not ideal
- ERPD – GF approved 3 pf-LDH RDTs manufactured by RapiGen ; these products are in WHO prequalification pipeline and passed lab evaluation
- Next generation pf-LDH RDTs in field trials this year

Product name	Product code(s)	Manufacturer name	Dossier review	On-site inspection	Laboratory evaluation
BIOCREDIT Malaria Ag Pf (pLDH)	C14RHG25 and C14RHH25	RapiGen Inc.	R		◆
BIOCREDIT Malaria Ag Pf (pLDH/ HRP Ⅱ)	C13RHG25 and C13RHH25	RapiGen Inc.	R		◆
BIOCREDIT Malaria Ag Pf/Pv (pLDH/pLDH)	C61RHG25 and C61RHH25	RapiGen Inc.	R		◆

R	🗁	◆	F	S
information requested from manufacturer	in process	stage complete	follow-up amendments	scheduled; date confirmed

Please note: these tables are updated regularly; while every attempt is made to provide current data, the most recent information might not be reflected. This table is intended only as an update on progress and does not reflect a final decision on prequalification. This table should not be used to inform procurement. Information may not yet be reflected here.

Last update: 2 October 2020

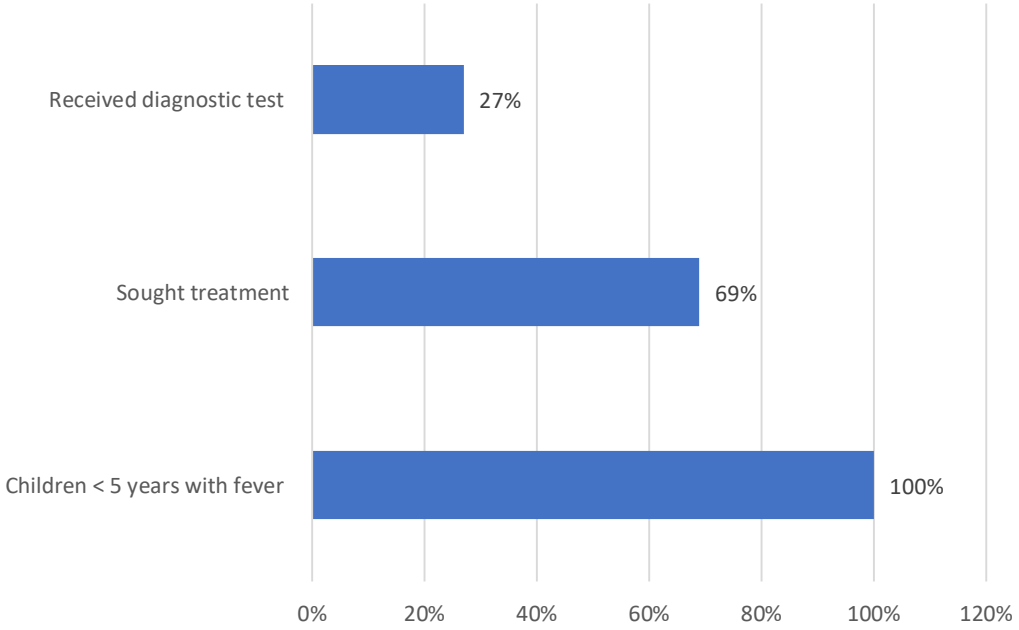
http://www.who.int/diagnostics_laboratory/pq_status/en/index.html

Supply security risk
Elevated price

World Health Organization

Other diagnostic challenges - access to quality diagnostic testing

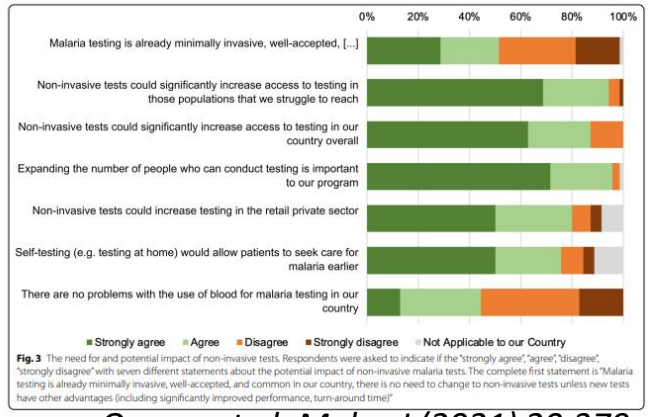
Base on HH surveys in 20 African countries – only a quarter of those who should be tested are being tested



Source: adapted from World malaria report 2021

Understanding and addressing barriers ….

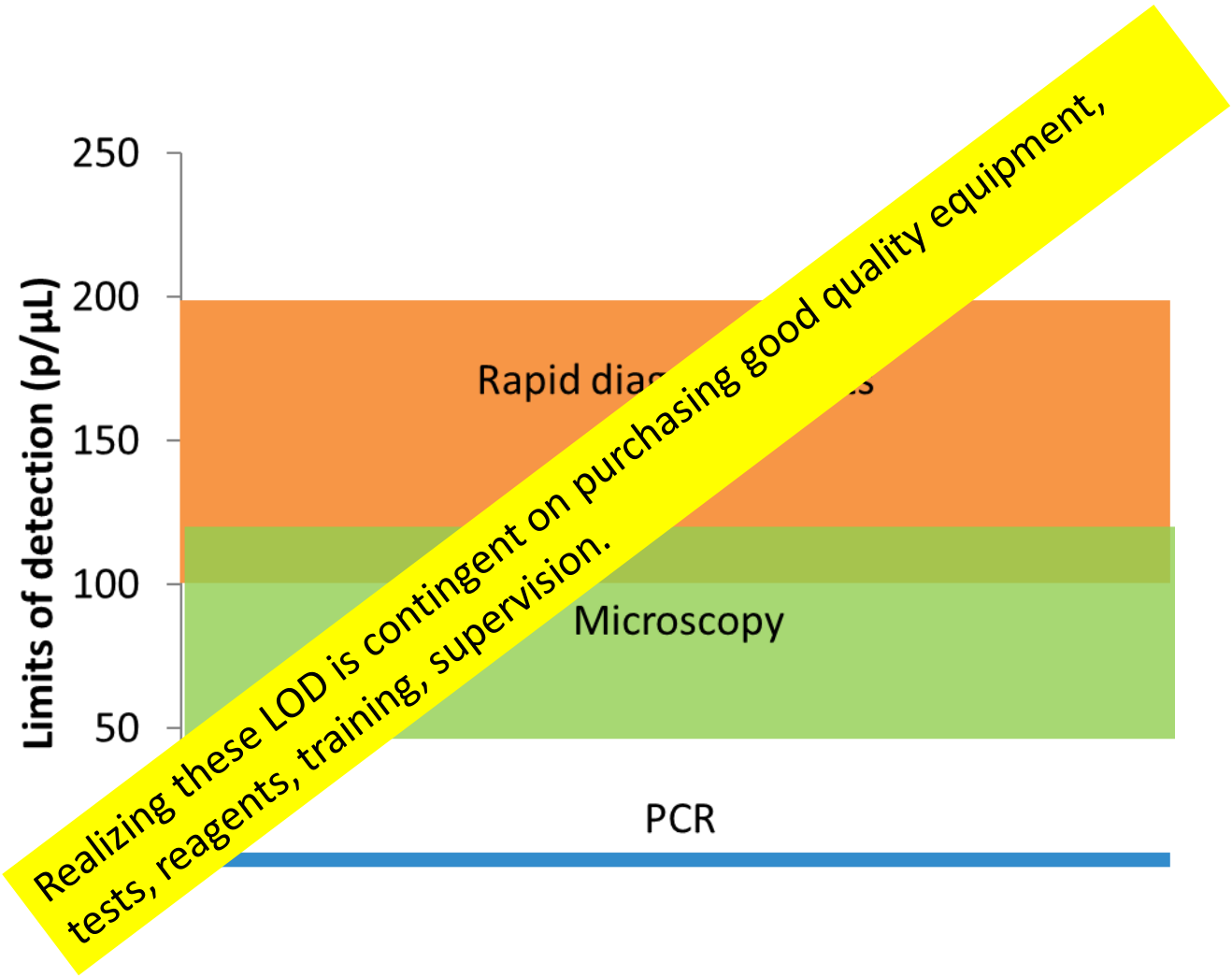
- Expansion of existing solutions
  - CHWs, iCCM
  - RDTs in pharmacies, drug vendors
- Exploring new solutions
  - Self-testing
    - https://www.malakitproject.org/overview/
  - Non-invasive testing



Owusu et al. Malar J (2021) 20:379

- Digital tools for data capture and better targeting of interventions and enhanced quality control

?? Low density infections – what is the target ?



1435 children NE Tanzania

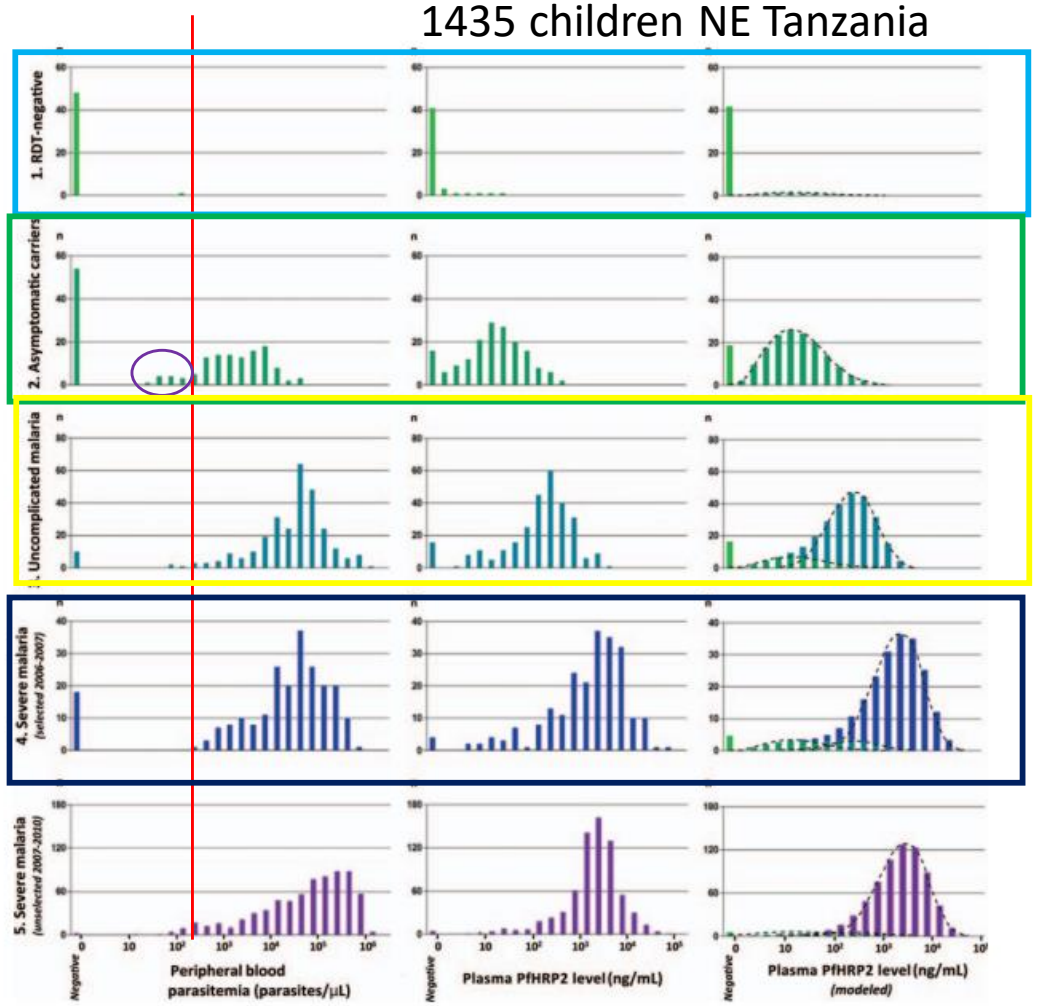


Figure 2. Frequency distributions of peripheral blood parasitemia, plasma *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2) concentrations, and modeled fitted *Pf*HRP2, according to malaria clinical group (1 = healthy rapid diagnostic test [RDT]–negative controls, 2 = asymptomatic carriers, 3 = uncomplicated malaria, 4 = severe malaria, 5 = severe malaria). The fitted *Pf*HRP2 distributions (right column) show the modeled *Pf*HRP2 distributions with the underlying contributing *Pf*HRP2 distributions of different diagnostic groups (dotted lines), composed of RDT-negative controls (light green), asymptomatic carriers (green), and patients with uncomplicated malaria (blue turquoise) or severe malaria (bright blue and purple).

Hendriksen et al • JID 2013:207

Conclusions

- Globally, there are hotspots for pfhrp2/3 deletions – South America and the Horn of Africa region, models predict other high risk areas in SSA
- Surveillance should be high priority for all countries particularly where pfhrp2/3 deletions have been reported locally or in neighboring countries – avoid crisis !
- With continued HRP2 RDT pressure expect the problem is ongoing and getting worse and areas where malaria is being driven down to low levels may be at higher risk
- Alternative tests that does NOT rely on HRP2 are available – in PQ pipeline and GF ERPD approved
- Removing barriers to access diagnosis could have a much bigger impact that detecting low density infections particularly in moderate, high transmission areas
- Realizing the full potential of diagnostics requires that all links in the chain are strong and maintained