MESA FORUM

Responding to the threat of malaria parasites evading HRP2-RDTs

PRESENTATIONS:

- Overview and relevance of *hrp2/3* gene deletion [D. Ishengoma] ---------- p.2
- *pfhrp2/3* deletion in *P. falciparum*: the experience of Peru [D. Gamboa] ----- p.7
- Malaria RDTs and *pfhrp2* deletion in Eritrea [S. Mihreteab] ---------- p.12
- Suspecting and tracking *pfhrp2/3* deletions [J. Cunningham] ---------- p.22
- Approaches for screening and confirming *pfhrp2/3* deletions --------- (not included)

Responding to the threat of malaria parasites evading HRP-2 RDTs

Overview and relevance of *hrp2/3* gene deletion

Deus S. Ishengoma
Research Scientist
National Institute for Medical Research

Malaria case Management

- Malaria case management depends on early and timely diagnosis + effective treatment with ACTs
- Case management is an important pillar of the ongoing malaria control and elimination strategies in all endemic countries
- High quality diagnostic services are critical for effective case management
- Malaria diagnosis has suffered from poor services due reliance on microscopy. Microscopy is limited by:
  - Inadequate skills of microscopists
  - Demand for functional and well-maintained microscopes
  - Poor and/or lack of high-quality reagents
  - Logistics and infrastructure: electricity, water, lab space etc

RDTs: The magic bullet?

- In 2010 WHO recommended use of RDTs which have greatly revolutionized malaria diagnosis especially in rural areas
  - Easy to use by staff even those with limited training
  - Provide results in a very short time, within 15 – 30 min
  - Can be stored at room temperature, no demand for expensive storage equipment
  - No demand for expensive equipment and lab space
  - The antigens used are stable and have good sensitivity
- RDTs have many limitations but still are the best option:
  - Persistence of HRP-2 antigens
  - Failure to detect low density infections
  - False results due to device errors caused by poor storage, poor interpretation, packaging, and transport conditions
  - Recent emergence and spread of *hrp2/3* gene deletion
- This webinar will discuss the emerging threat of hrp2/3 gene deletion and how to contain/manage this crisis

Presentations and presenters

- **Dr. Dionicia Gamboa:** is a biologist and associate professor at Universidad Peruana Cayetano Heredia, Peru. Her current research focuses on characterizing malaria transmission in rural communities in the Peruvian Amazon. In 2010, Dr. Gamboa reported for the first time *pfhrp2* gene deletion in *P. falciparum* in a clinical setting of Iquitos, Loreto. Today, she will share with us an **overview of how Peru has been facing this challenge** since then.
- **Dr. Selam Mihreteab:** is the manager of the NMCP in Eritrea since 2012. Eritrea is the first African country to complete a nationwide switch away from HRP2-based RDTs due to high prevalence of the deletions. Dr. Mihreteab was one of the focal persons involved in the national response to this new challenge and today, he will share **his experience** with us.
- **Dr. Jane Cunningham**: is a Medical Officer at the Global Malaria Programme of WHO in Geneva. She coordinates development of malaria diagnostic guidance and related activities . Today she will present us an overview on '**Suspecting and tracking *pfhrp2/3*-deletions**.
- **Dr. Eric Rogier**: is a microbiologist within the Division of Parasitic Diseases and Malaria at CDC in Atlanta, USA. His laboratory works to develop high-throughput laboratory assays for markers of malaria exposure and is one of the key members of the "WHO international Lab Network to support *pfhrp2/3*-deletion surveillance". Today he will present us an overview of the **approaches for screening and confirming *pfhrp2/3*-deletions**.

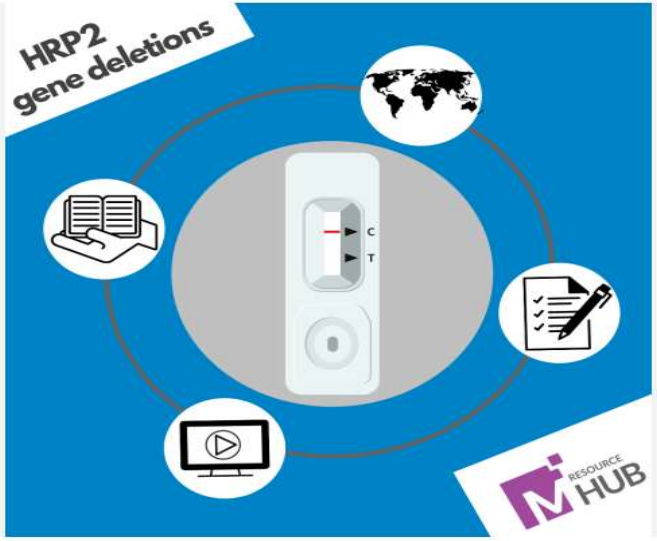
Acknowledgments & Resources

Organizers

MESA

Last Updated 12 May 2022

Resource compilation: Responding to the threat of pfhrp2/3 deletions



http://www.mesamalaria.org/resource-hub/resource-compilation-responding-threat-pfhrp23-deletions

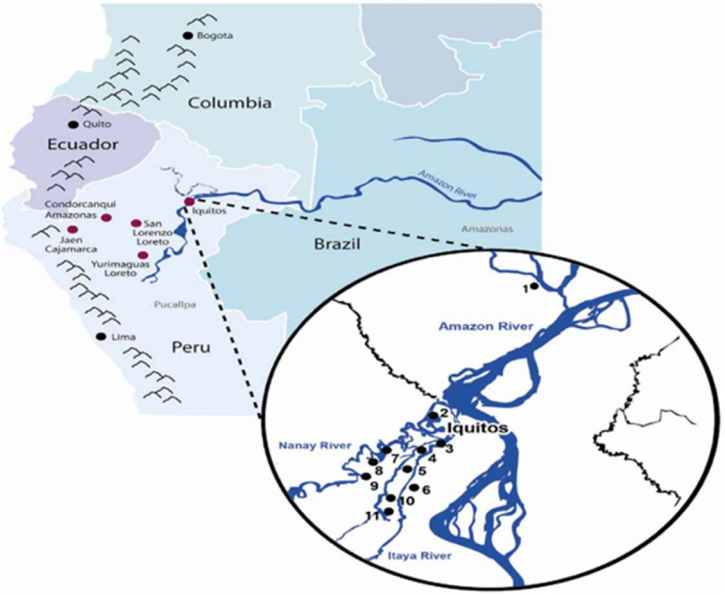
UNIVERSIDAD PERUANA
CAYETANO HEREDIA

pfhrp2/3 deletion in *Plasmodium falciparum*: the experience of Peru

Dionicia Gamboa Vilela, PhD
Associate professor & Head of the Malaria Laboratory

June 14th, 2022

pfhrp2/3 deletion in Peru and South America



Peru
Loreto (2003-2008),
148 samples

41% *pfhrp2* (-)
70% *pfhrp3* (-)
21.6% *pfhrp2/pfhrp3* (-)

Gamboa et al, PLOS ONE 2010

Colombia
1999-2009
18% pfhrp2-
52% *pfhrp3-*
C Murillo et al, PLOS ONE 2015
2003-2010
5.9% pfhrp2-
41.9% *pfhrp3-*
2011-2012
45.5% *pfhrp3-*
EJ Dorado et al, PLOS ONE 2016

Perú
1998-2001
20% *pfhrp2-*
2003-2005
40% *pfhrp2-*
S Akinyi et al, Sci Rep. 2013
2012-2014
53% *pfhrp2-*
48% *pfhrp3-*
37% *pfhrp2-/pfhrp3-*
Quispe Carbajal, 2017 (MSc thesis)

Suriname
2009-2011
14% *pfhrp2-*
4% *pfhrp3-*
2.6% *pfhrp2-/pfhrp3-*
S Akinyi et al, PLOS ONE 2015

Brazil
2011-2012
13.6% *pfhrp2-*
35.9% *pfhrp3-*
11.6% *pfhrp2-/pfhrp3-*
GM Rachid Viana et al, PLOS ONE 2017
2016-2017
71.7 -100% *pfhrp2-*
94.9 - 98.3% *pfhrp3-*
79.8% *pfhrp2-/pfhrp3-*
L Goes et al, Int J Environ Res Public Health 2021

Apparently the frequency of *P. falciparum pfhrp2/pfhrp3* negative has increased over time, why?

Malaria diagnosis in Peru

- In Peru: *P. vivax* (~70%) and *P. falciparum* (~30%)
- <u>Microscopy:</u>
  - Laboratory technicians
    - 2007-2010: ~300 microscopists trained (PAMAFRO project)
    - 2018-2021: 241 microscopists trained within the **Malaria Zero Plan-MZP** (Elimination plan from the Peruvian MoH) + 25 for international certification by National Institute of Health (INS)
    - Evaluation by INS: every trimester (performance) and each semester (competences)
- <u>Rapid diagnostic tests</u> (RDTs): based on HRP2 and/or LDH
  - Laboratory technicians
  - Community health promoters (ACS), they are trained to use RDTs and provide treatment:
    - 1222 ACS trained within the MZP: 60% indigenous, 80% male, 75% only with primary school

Technical documents for diagnosis from INS, MoH

Training activities for ACS and microscopists

*Data and pictures provided by Dr. Hugo Rodriguez (MZP coordinator for Loreto region)

Use of RDTs in Peru

- <u>Before *pfhrp2* deletion</u>
  - Based on Pf HRP2 and Pan LDH or Pv LDH
  - 2-band RDT
- Easy to use and interpret

- <u>After *pfhrp2* deletion</u>
  - Based on Pf HRP2, Pf LDH and Pv LDH
  - 3-band RDT
- Difficult at the beginning, now we are used to this RDT
- New challenges:
  - Very low parasitemia
  - Asymptomatic infections
- New tools:
  - Other markers
  - Ultra sensitive RDTs

¡Gracias!

Malaria Zero Plan, Peruvian Minister of Health
CDC (Venkatachalam Udhayakumar)
Foundation for Innovative New Diagnostics (FIND)

Malaria RDTs and *Pf*hrp2 Deletion in ERITREA

14 June 2022

OUTLINE

- Introduction
- Initial Investigation of reported false-negative RDT Results
- Confirmatory Investigation
- Lessons Learnt/Recommendations

INTRODUCTION

- Diagnosis policy in Eritrea – All suspected malaria cases must be parasitologically tested (Mic or RDT);
- ~ 75% of suspected malaria cases are diagnosed using RDTs (lower-level facilities and Community level);
- Eritrea has been using HRP2-*Pf* /pLDH-*Pv* Combo RDTs since 2006;
- A number of RDT quality defect (false-negative RDT results but +Mic) complaints reported to Pharmacovigilance Center (2014-15) for SD Bioline Malaria Ag Pfhrp2/Pv-LDH (05FK80). This is for patients strongly suspected as Malaria

preliminary investigation

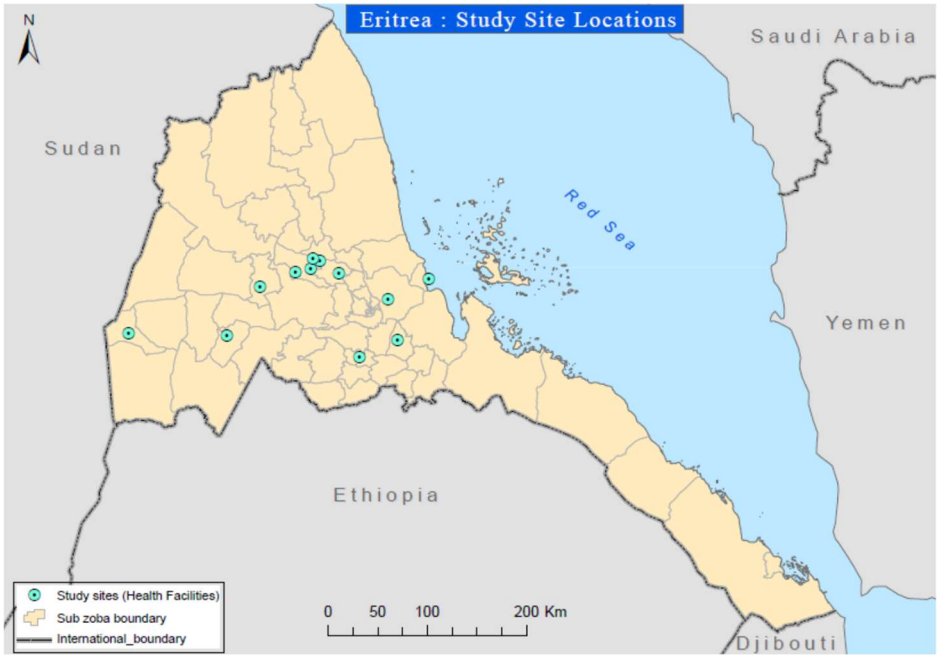
INITIAL INVESTIGATION

- WHO user complaint form for reporting problems on diagnostic products submitted;
- Investigations…

INVESTIGATIONS	RESULTS
Storage facilities	Standard
Transportation of products and Operator condition	Optimal
Performance of RDTs by External Lab	QC Passed
Investigation by SD Company	False negativity of *P.f* cases confirmed
Performance of SD Bioline Malaria Ag Pf/Pv (05FK80) against Mic. (12 HFs)	82% False negative rate (for *P.f*)
	Product recalled from market – Jan 2016

INITIAL INVESTIGATION – MOH

Initial comparative results of SD Bioline Malaria Ag Pf/Pv & Microscopy			
	P. falciparum		
Region	**Mic+**	**HRP2 test line negative**	**%**
NRS	12	12	100
Anseba	14	13	92.9
Gash Barka	17	11	64.7
Debub	7	5	71.4
TOTAL	**50**	**41**	**82.0**



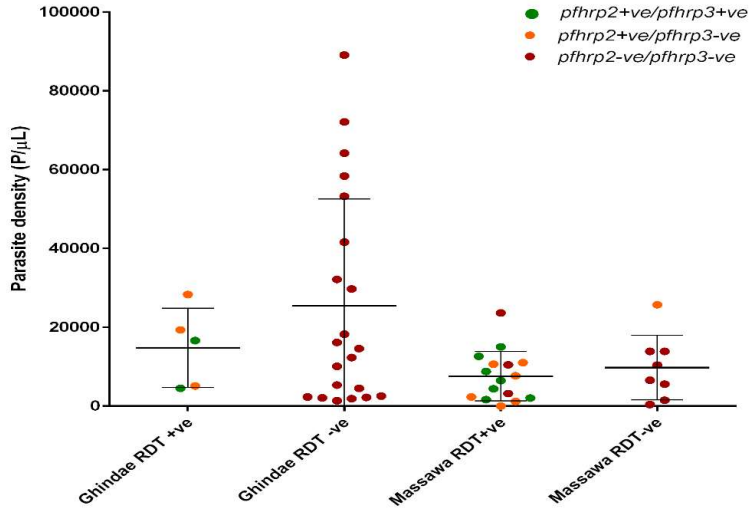
CONFIRMATORY INVESTIGATION: MOH-WHO-Other partners

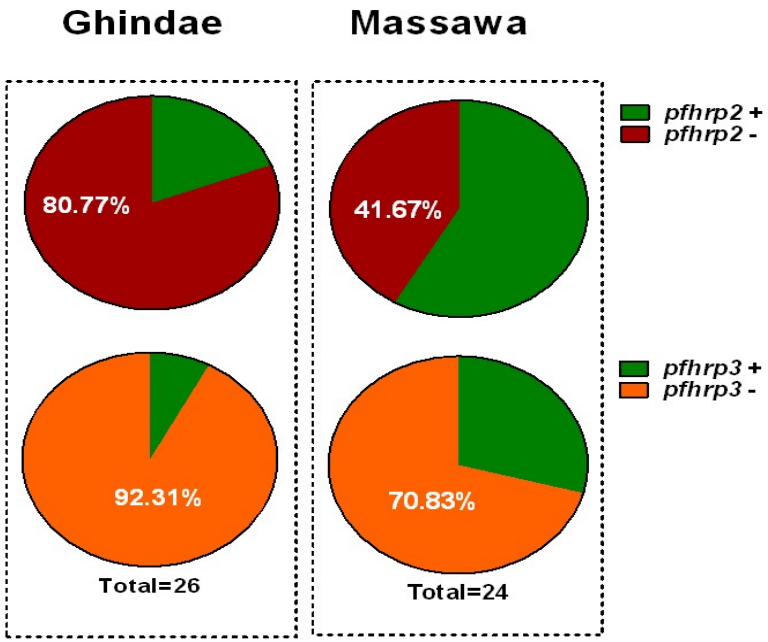
- Consecutive malaria suspects (N=50) screened in 2 hospitals with HRP2 & non-HRP2 based RDTs: Jan-Feb 2016
  - Carestart™Malaria pLDH(PAN) G0111 and SD Bioline Malaria Ag Pf/Pf/Pv; 05FK120; and Micros.
  - DBS

INVESTIGATIONS	RESULTS
Microscopy vs diff. types of RDTs	**All** specimens reacted to PAN-only RDTs (pLDH) & **62%** of Mic.+ve *P.f* specimens tested negative with *pf*HRP2-RDTs
PCR-analysis of specimens	Mic. and PCR results matched 100% *(species confirmation)*
Characterization of *pf*hrp2 sequences	Absence of *pf*hrp2 genes confirmed
Luminex mulitplex bead assay	Assay re-confirmed deletion of *pf*hrp2 gene, i.e All PCR *pfhrp2* negatives sample had undetectable HRP2 antigen levels

RESULTS – *Molecular Analysis*

Study Site	BF +ve (*P. f* cases)	HRP2_Exon 1 & 2 Deletion	
		No.	%
Ghindae	26	21	80.8
Massawa	24	10	41.7
Total	**50**	**31**	**62.0** [95% C.I: 55-69]





Challenges

- No parasitological diagno. for 1 yr.
  - Patients refusing treatment without blood testing when RDTs were recalled
  - Increase in clinically diagnosed and reported malaria cases – difficult to get the real malaria situation in 2016
  - Overconsumption of antimalarial drugs
  - Malaria cases might have been missed
- Few non- HRP2-based RDT options, are less sensitive…
  - Eritrea requires RDTs detecting *Pf* and *Pv*.
- Switching to new RDTs several times [Pf/Pan (pLDH/pLDH)] → Combination RDTs (Pan-pLDH + Pfhrp2/Pv-pLDH) → Pf/Pv (pLDH/pLDH)
- The need to re-train staff on the new RDTs…

LESSONS LEARNT & RECOMMENDATIONS

1. Being vigilant for false-negative or product defect complains is crucial
2. In case of the need to change RDTs, prior testing at field conditions is helpful
3. Regular surveillance of RDT performance (QA/QC);
4. Promote R&D of non-HRP2 based diagnosis;

ACKNOWLEDGEMENT

- Study subjects
- Study sites (HFs) and the zones
- CDC, MOH
- Pharmacovigilance Center, MOH
- National Health Lab, MOH
- WHO
- Australian Army Malaria Institute
- CDC-Atlanta

Suspecting and tracking pfhrp2/3 deletions

Jane Cunningham, Medical Officer

cunninghamj@who.int

14 June, 2022, MESA FORUM

Global **Malaria** Programme

World Health Organization

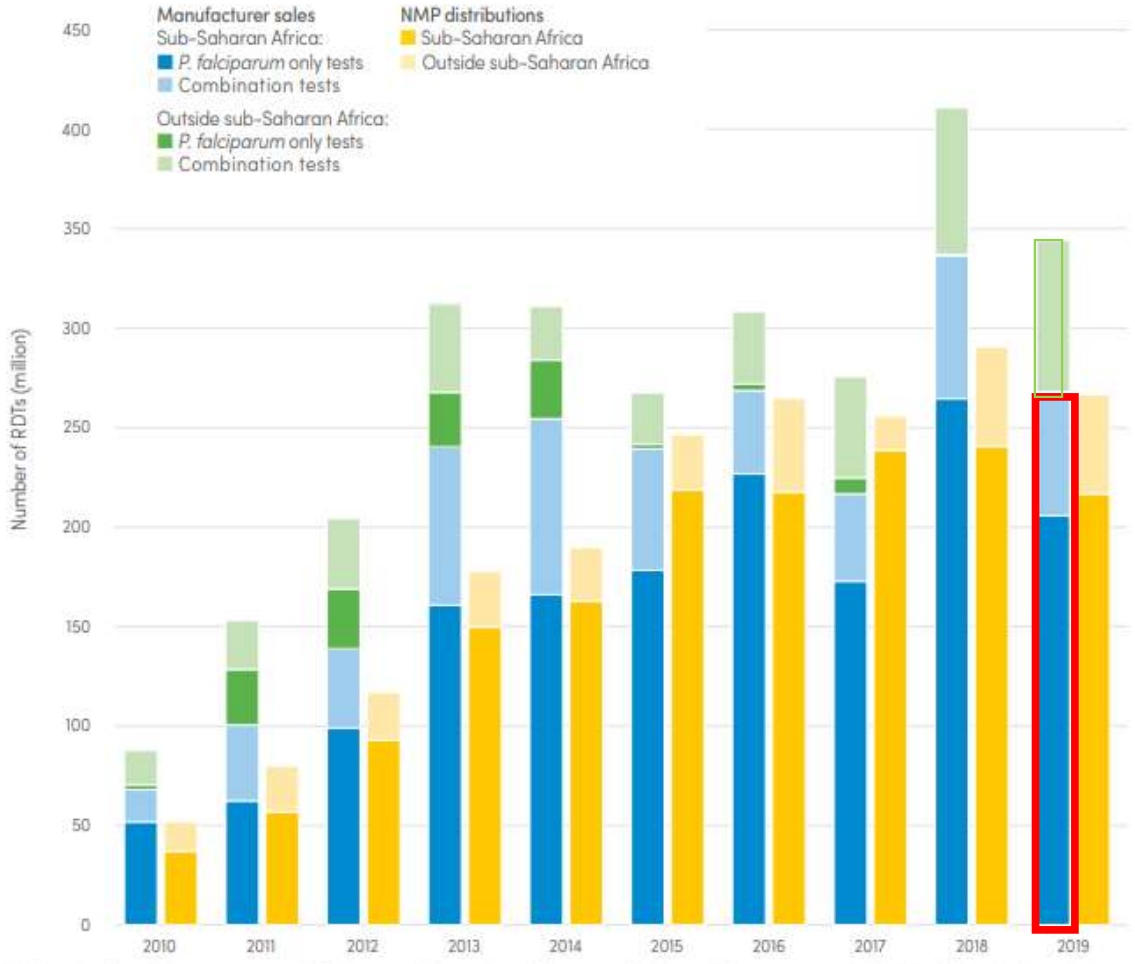
Background

- RDTs target a range of malaria antigens

	HRP2	pLDH	Aldolase
***P.falciparum*-specific**	+	+	
Pan-specific (all species)		+	+
***P.vivax*-specific**		+	

- The majority of RDTs used to detect *P. falciparum* target histidine rich protein-2

Number of RDTs sold by manufacturers and distributed by NMPs for use in testing suspected malaria cases, 2010–2019[a] *Sources: NMP reports and sales data from manufacturers eligible for the WHO Malaria RDT Product Testing Programme.*



NMP: national malaria programme; *P. falciparum*: *Plasmodium falciparum*; RDT: rapid diagnostic test; WHO: World Health Organization.
[a] NMP distributions do not reflect those RDTs still in storage that have yet to be delivered to health facilities and community health workers.

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

Proactive surveillance – if reports in country or neighbors

Two templates available approved by WHO ERC:

Focus on suspected malaria cases and "false"negative RDT results -- underestimates prevalence of *pfhrp2/3* deletions BUT identifies CLINICALLY RELEVANT deletions

- **Protocol for Surveillance** (only)

All suspected malaria cases tested simultaneously with:

2 RDTs: HRP2 ("program") & pf-LDH* ("survey")

OR

1 RDT + MIC: HRP2 ("program") & Microscopy

AND

2 Dried Blood spots (collected)

RESULTS of parallel testing:

- Discordant samples (HRP2- & pf-LDH+ // HRP2- & Mic+) prioritized for molecular analysis

- If resources available, include a subset of other samples for molecular analysis

- **Protocol for Surveillance + Biobanking**:

Involves asking consent for long term storage of samples -> If yes, samples are kept to support future research

Global Malaria Programme

**pan-LDH is alternative but will detect non-P.falciparum cases – increased discordance*

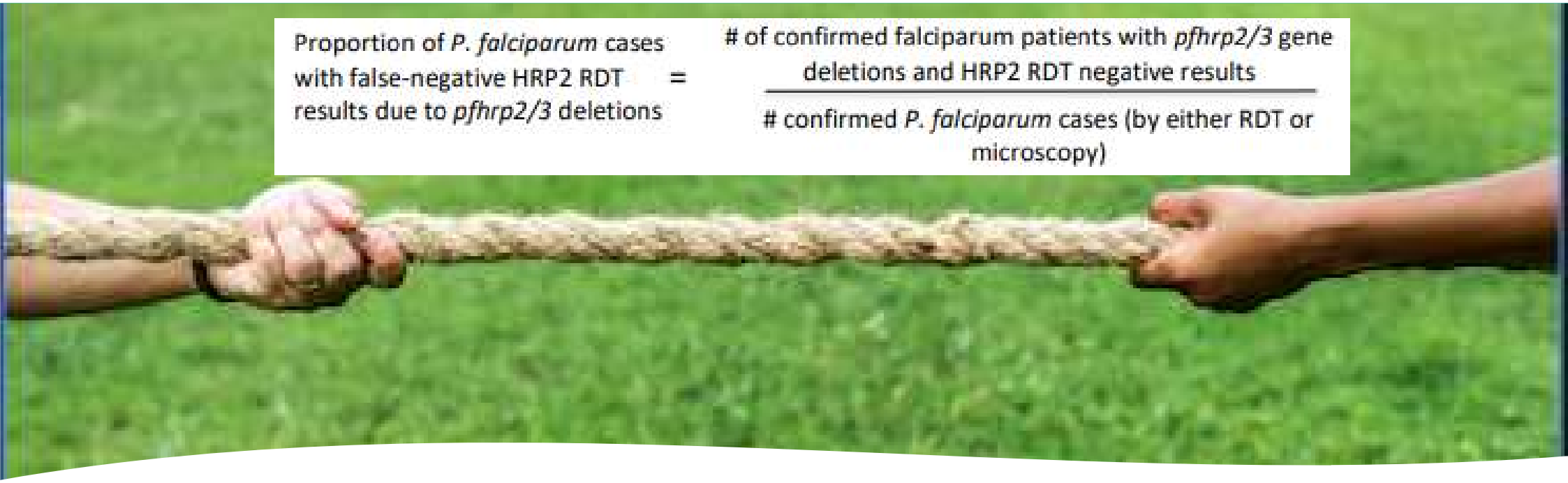
World Health Organization

WHO international Lab Network to support pfhrp2/3 surveillance

- Set of geographically diverse labs with experience characterizing pfhrp2/3 deletions and participating on the WHO NAAT EQA
- Terms of reference
- Engage in tripartite agreements between WHO-Lab-survey country (MOH, research institute)
- WHO has some funding to support molecular and sero analysis and some of the labs also have funding sources
- Contact WHO to be directed to a lab

Contact person	Location Country	Institute	Contact details
Dr. Khalid Bashir/ Dr. Colin Sutherland	UK	Medical Research Laboratories/ London School of Hygiene and Tropical Medicine	Khalid.Beshir@lshtm.ac.uk
Dr. Jonathan Parr	USA	University of North Carolina	jonathan.parr@unchealth.unc.edu
Dr. Qin Cheng	Australia	Australian Defence Force Malaria and Infectious Disease Institute (ADFMIDI, formerly AMI)) and QIMR-Berghofer Medical Research Institute	Email: qin.cheng@defence.gov.au Tel: +61-7-3332 4834 Fax: +61-7-3332 4800 Skype : qin543211
Dr. Venkatachalam Udhayakumar/Eric Rogier	USA	Centres for Disease Control	vxu0@cdc.gov
Professor Daouda Ndiaye	Senegal	Université Cheikh Anta Diop de Dakar (UCAD)	daouda.ndiaye@ucad.edu.sn
Dr. Dionicia Gamboa	Peru	Universidad Peruana Cayetano Heredia	dionigamboa@yahoo.com
Dr. Praveen Bhatri	India	NIMR-National Institute of Malaria Research(India)	saprapbs@yahoo.co.in

Will be expanding the network in 2022-2023 – get in touch and join WHO NAAT EQA scheme: MalNAATEQA@who.int



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

Surveillance protocol – 5% threshold – above or below

Sampling

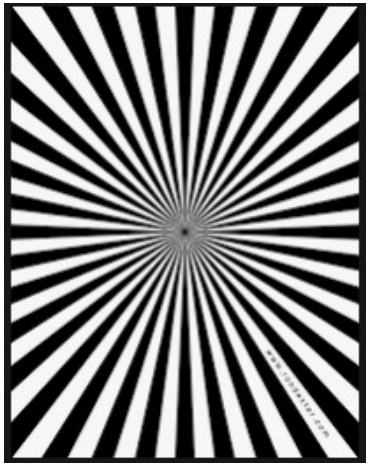
- Suggested sample sizes per domain are based on an estimated percentage of 3.4% or 7.2% confirmed pfhrp2 deletions causing FN HRP-RDTs
- If the true percentage is < 3.4% or > 7.2% the SS requirements will be less.
- The closer the true value is to 5% the greater the SS needed to determine if truth is > or < 5% with 95% confidence
  - Not feasible in most cases - plan to repeat survey in 1-2 yrs.
- Within the domain chosen – 10 health facilities (37 Pf cases/ HF) selected on the basis of probability proportional to size depending on the fever or suspected malaria caseload
- Cover all transmission zones

Percentage of confirmed *pfhrp2* deletions causing false negative HRP2 RDT results	Minimum number of individuals with confirmed P. falciparum infection to include per domain, to estimate sample size needed to ensure the 95% confidence interval (1-tailed test) does not include 5% prevalence of *pfhrp2/3* deletions
%	n
3.0	205
3.2	260
3.4	369
3.6	487
3.8	757
4.0	1,082
4.2	2,016
4.4	3,484
4.6	11,133
4.8	32,202
5.0	
5.2	34,739
5.4	10,240
5.6	4,379
5.8	2,457
6.0	1,590
6.2	1,123
6.4	841
6.6	658
6.8	531
7.0	459
7.2	386
7.4	331
7.6	287
7.8	253
8.0	224
8.2	205

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.**

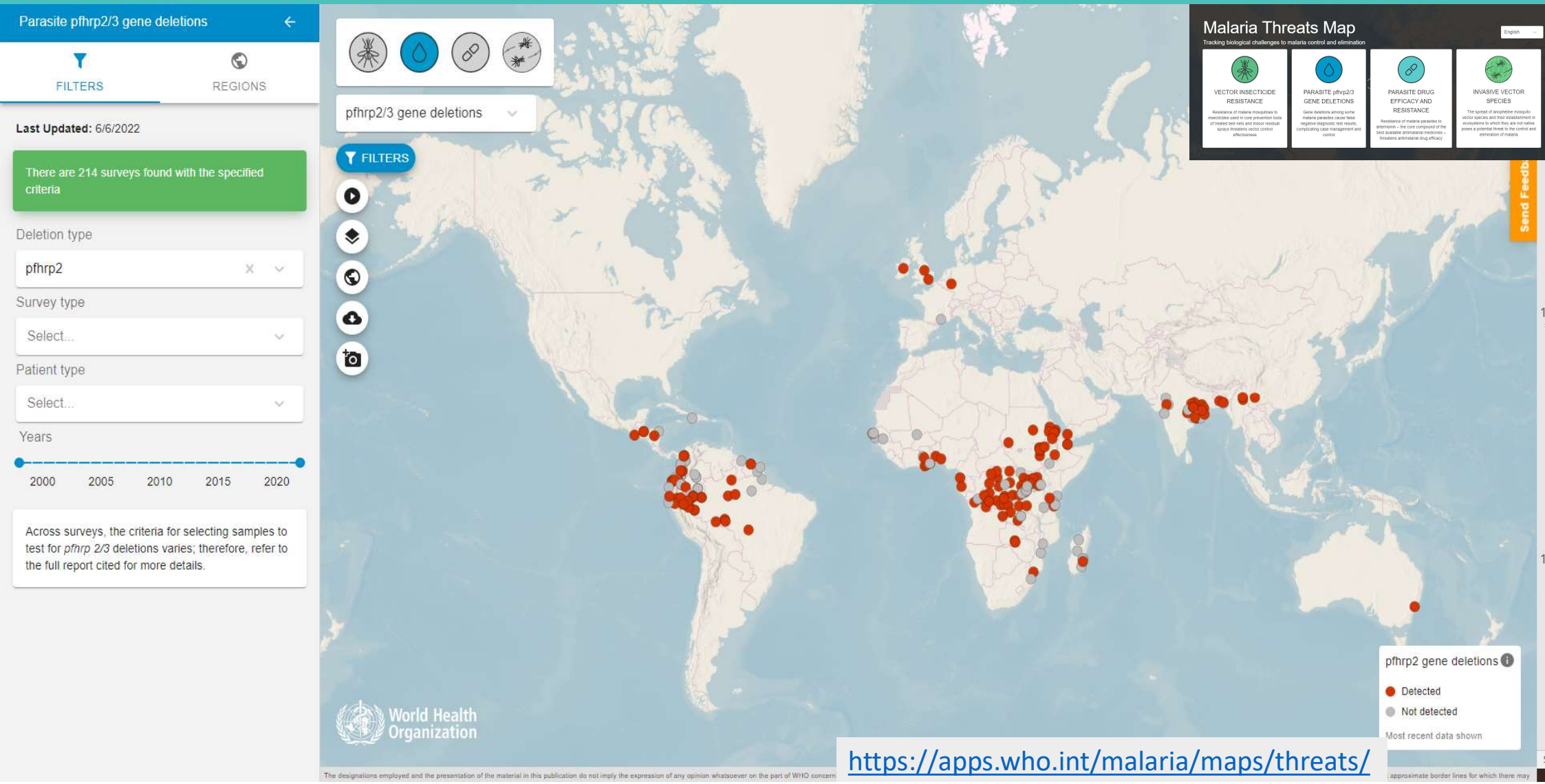
WHO Response Plan to *pfhrp2/3* deletions

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

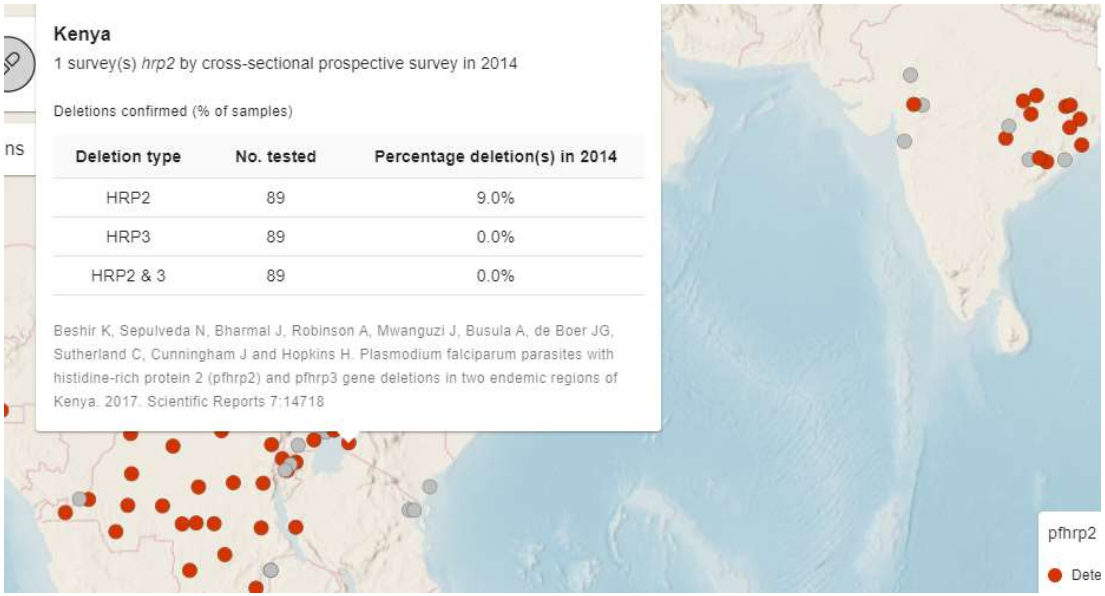
How do we track ? WHO Malaria Threat Maps



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>



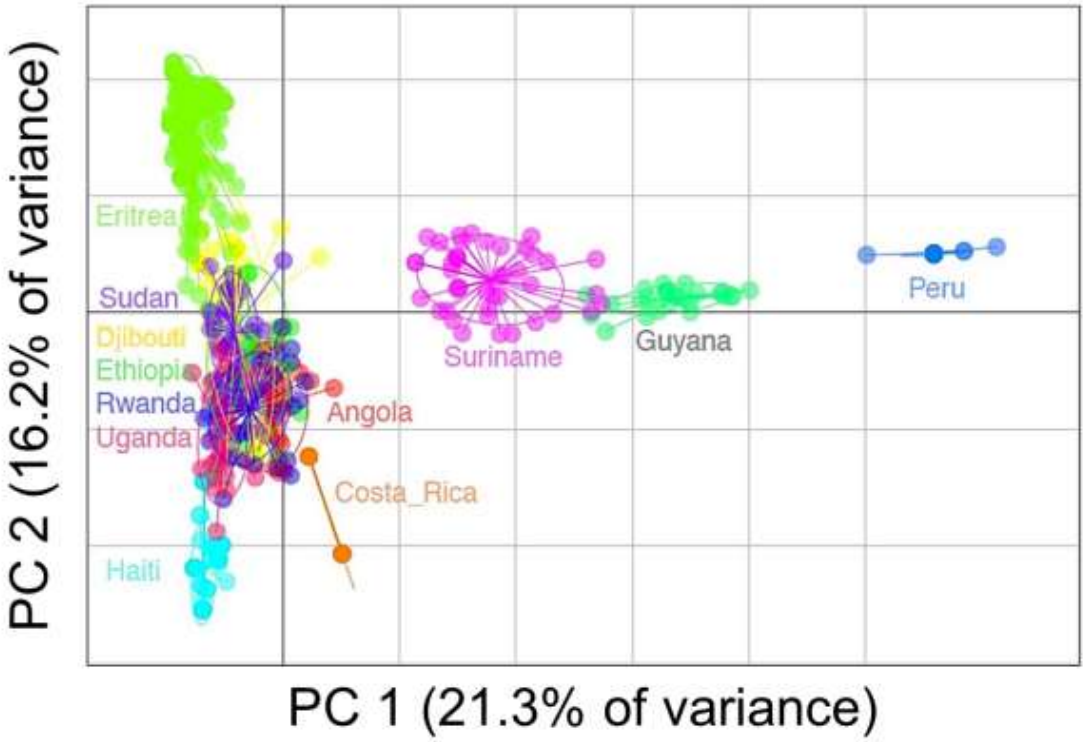
Way forward – complementary dashboard of planned and ongoing surveys; indicate where RDT policy has changed

Are pfhrp2/3 deletions spreading or emerging ….BOTH

15

Global *P. falciparum* Relatedness

Regardless of *pfhrp2/3* Genotype



Clear clustering by geographical location

Djibouti parasites look very African

Courtesy: Eric Rogier, US CDC, manuscript in preparation

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

Conclusions

- Health providers and NMCPs need to be aware and responsive to threat of pfhrp2/3 deletions without undermining confidence – “get ahead of the curve”
- Strengthen communication for reporting problems and implement surveillance
- Use WHO protocol templates to develop surveys that are designed and powered to inform policy change.
  - Surveillance approach and using existing health workforce <<< expensive than research
- With continued HRP2 RDT pressure expect problem to grow
  - need more historical data and research
- An alternative RDTs not entirely reliant on HRP2 for Pf detection are limited but available (in PQ pipeline and GF ERPD approved) and more going into field trials in 2022 combo test that does rely on HRP2 is available

Resources

Last Updated 12 May 2022

Resource compilation: Responding to the threat of pfhrp2/3 deletions

- http://www.mesamalaria.org/resource-hub/resource-compilation-responding-threat-pfhrp23-deletions

World Health Organization