MESA FORUM

Traduction simultanée en français

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

3 April **2pm CET** **Virtual**

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

Register today

and become a member of the CoP

@MESAmalaria

MESA FORUM

Streamed live via the MESA YouTube channel

Duration

90 minutes

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

Co-Chairs

Deus Ishengoma
IHI - NIMR – KIUT, Tanzania

Nana Aba Williams
MESA

Panelists

Jane Cunningham
WHO/GMP

Regina Kandie and Hosea Akala
NMCP-MoH, Kenya

Matthew Coldiron
MSF, South Sudan

1. **Introduction** *5 min*
   Deus Ishengoma
2. **New WHO guidance documents** *20-25 min*
   Jane Cunningham
3. **Experience from the field - Recent surveys** *20-30 min*
   Regina Kandie, Hosea Akala and Matthew Coldiron
4. **Discussion and Q&A** *25 min*
5. **Closing remarks** *5 min*
   Deus Ishengoma

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Responding to threat of pfhrp2/3 deletions
WHO updates

MESA Community of Practice Forum
3 April 2025

Dr Jane CUNNINGHAM
Diagnostics, Medicines and Resistance Unit

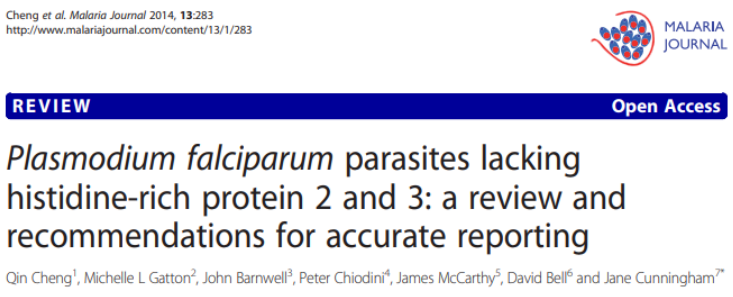
Global **Malaria** Programme

World Health Organization

Overview

- Response plan for *pfhrp2* deletions - Update and core actions
- Surveillance - Protocol revisions
- Other guidance:
  - Threshold for changing RDT
  - Current and future RDT options

Monitoring and responding to pfhrp2 deletions

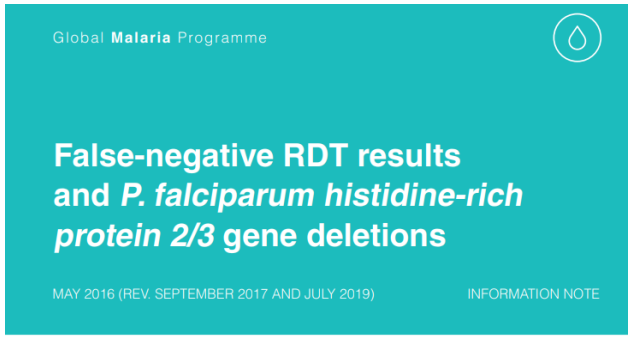

Cheng *et al. Malaria Journal* 2014, **13**:283
http://www.malariajournal.com/content/13/1/283

MALARIA JOURNAL

REVIEW **Open Access**

Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting

Qin Cheng[1], Michelle L Gatton[2], John Barnwell[3], Peter Chiodini[4], James McCarthy[5], David Bell[6] and Jane Cunningham[7*]


Global **Malaria** Programme

False-negative RDT results and *P. falciparum histidine-rich protein 2/3* gene deletions

MAY 2016 (REV. SEPTEMBER 2017 AND JULY 2019) INFORMATION NOTE


Response plan to *pfhrp2* gene deletions

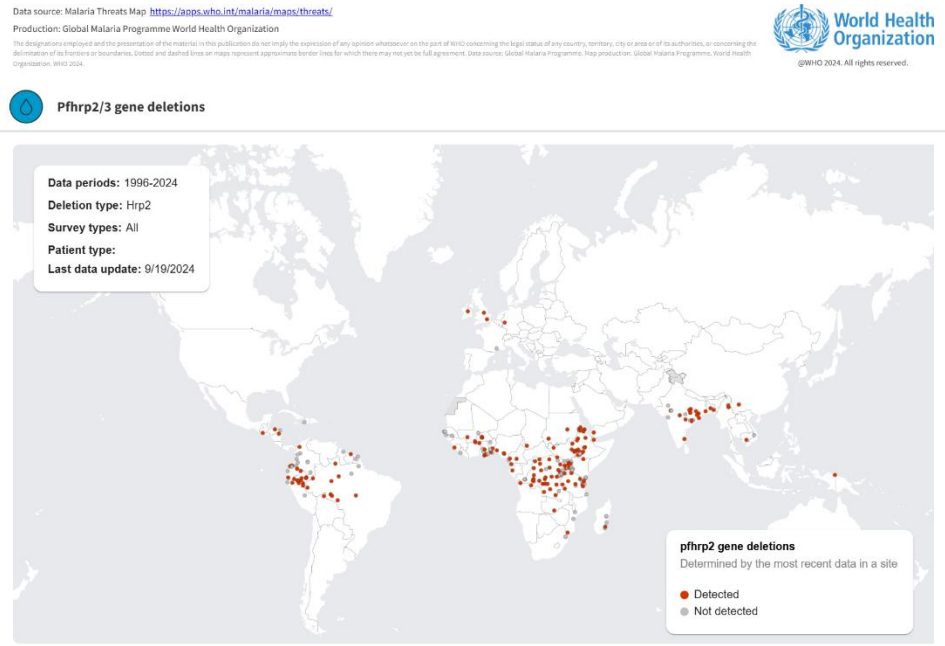
Second edition

World Health Organization

World Health Organization

https://iris.who.int/bitstream/handle/10665/379469/9789240101838-eng.pdf?sequence=1

Malaria Threat Maps – plans and reports

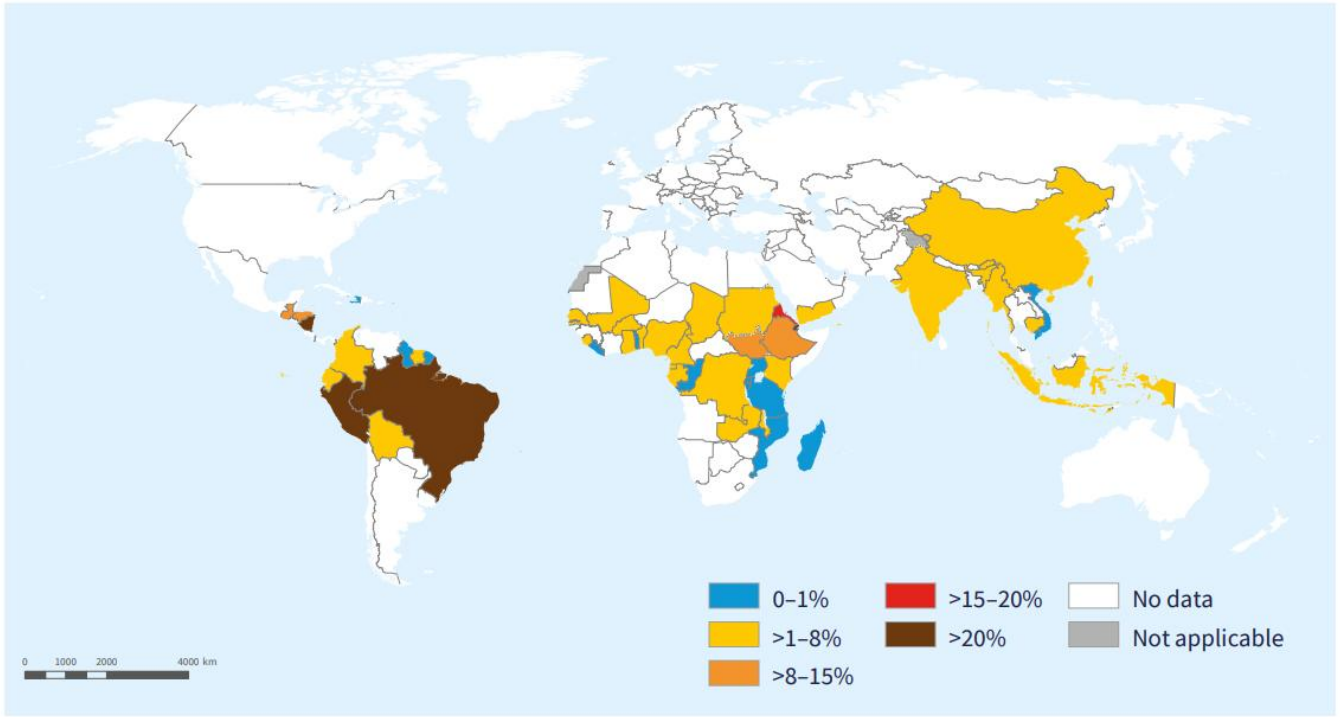


https://www.who.int/teams/global-malaria-programme/surveillance/malaria-threats-map

HRP2 gene deletions continue to emerge and spread, threatening lives of malaria patients

- **Parasites with genetic mutations that prevent diagnosis:** As of 2023, malaria parasites with *pfhrp2* gene deletions had been reported in 41 endemic countries.
- Four countries reported *pfhrp2* gene deletions for the first time in 2023: Burkina Faso, Chad, Togo and Indonesia.

Fig. 8.1. Estimated prevalence of *pfhrp2* gene deletions, 1996–2023 *Source: Review of published literature included in the Malaria Threats Map (69).*

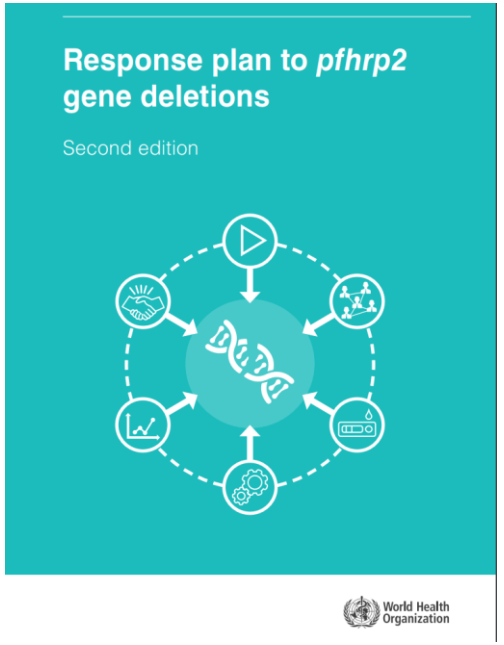


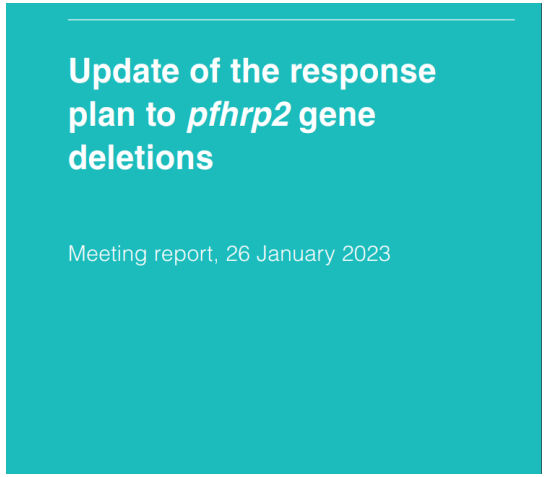
WMR, 2023

>400 million RDTs/ year; 90% in SSA and nearly all detect P. falciparum Based on HRP2

Core response plan actions

- Mapping the distribution and frequency of *pfhrp2/3* deletion mutants with harmonized protocols;
- Careful selection and procurement of new RDTs when a change of testing is warranted;
- Strengthening an international network of laboratories to perform the complex molecular confirmation required for mapping and identify new and/or efficient screening methods;
- Encouraging and facilitating research and diagnostics R&D
- Future planning with modelling





World Health Organization

https://iris.who.int/bitstream/handle/10665/378767/9789240097919-eng.pdf?sequence=1

WHO Recommends Prequalified RDTS …but they rely on HRP2

Table 3. WHO-recommended malaria RDT options for detecting both HRP2-expressing and non-expressing *P. falciparum* malaria for *pfhrp2/3* gene deletion surveillance

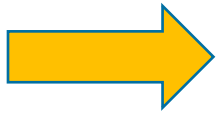
Performance criteria	A: *P. falciparum* PDS[a] ≥ 75% at 200 parasites/µL
	B: *P. vivax* PDS[a] ≥ 75% at 200 parasites/µL
	C: false-positive (FP) rate against clean negatives < 10%
	D: invalid rate (IR) < 5%
	E: *pfhrp2*-negative *P. falciparum* PDS > 75% at 200 parasites/µL (in areas where *pfhrp2* deletions are prevalent)

Table 4. Available non-WHO-prequalified tests meeting critical criteria[a]

Product name	Product code	Manufacturer name
Biocredit Malaria Ag Pf (pLDH)	C14RHG25, C14RHH25	Rapigen Inc.
Biocredit Malaria Ag Pf (pLDH/HRP2)	C13RHG25, C13RHH25	Rapigen Inc.
Biocredit Malaria Ag Pf/Pv (pLDH/pLDH)	C61RHG25, C61RHH25	Rapigen Inc.
CareStart™ Malaria Pf (HRP2/pLDH) Ag RDT	RMPM-02571	Access Bio Inc.
CareStart™ Malaria PAN (pLDH) Ag RDT	RMNM-02571	Access Bio Inc.

[a] Valid ISO 13485:2003, submission of application for WHO prequalification, and acceptable diagnostic performance against both HRP2-expressing and non-HRP2-expressing at 200 parasites/µL (*pfhrp2/3* single or double deletions), based on the most recent WHO laboratory assessment performed at the United States Centers for Disease Control and Prevention.

ERPD approval ; PQ reinspection Jan 2025



No longer have WHO prequalification (or ERPD)

**For surveillance purposes some countries have used – Standard Diagnostics 05FK90 or 05FK120 – with pfLDH test lines. These will miss lower parasitemias (200-1000p.uL)*

World Health Organization

https://www.theglobalfund.org/media/wwoh0ze0/psm_qadiagnosticsmalaria_list_en.pdf

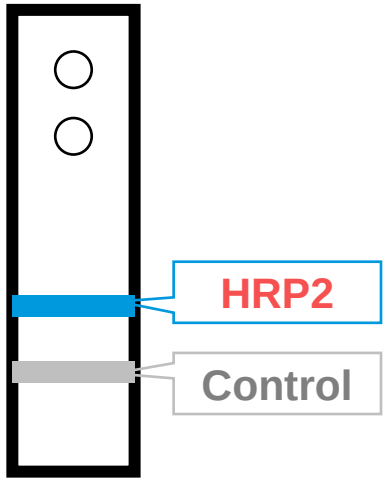
https://extranet.who.int/prequal/vitro-diagnostics/prequalified/in-vitro-diagnostics

New RDTs, have improved PfLDH, and HRP2 and PfLDH on the same RDT, are coming to market

New(er) RDTs

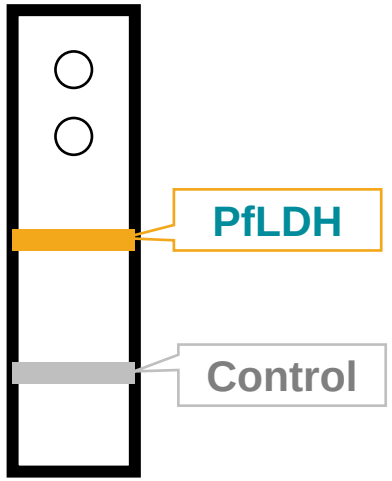
Next Gen RDTs

Status quo HRP2-only RDTs



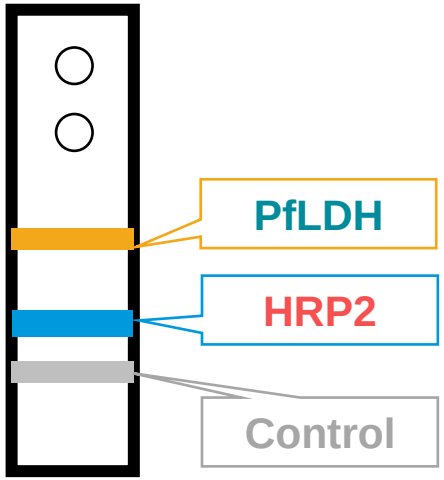
- Standard of care
- Cannot detect infections with *hrp2/3* gene deletions

PfLDH-only



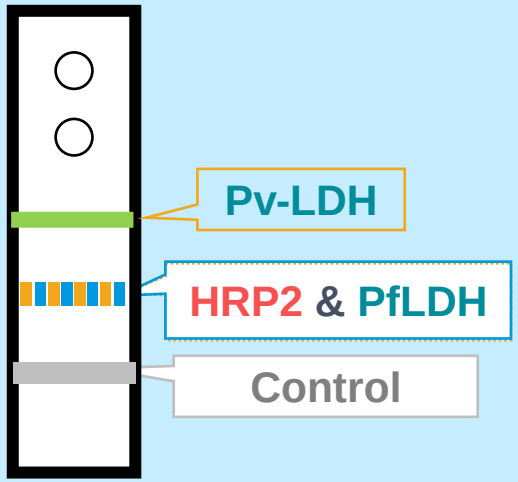
- ERPD approved
- Sensitivity trade-off

PfLDH and HRP2 separate line



- ERPD approved
- No-sensitivity trade-off
- Combines high sensitivity of HRP2 in non-deletion areas, and next gen PfLDH to detect infections with *hrp2* deletions

PfLDH and HRP2 combo line +- pv-LDH



- ERPD expected Q2 2025

RDTs with PfLDH and HRP2 can tackle both the challenge of *hrp2/3* deletions and the sensitivity trade-off between HRP2-only RDTs and PfLDH-only RDTs

WHO prequalification IVD malaria Pipeline

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: 4 March 2025
https://extranet.who.int/pqweb/vitro-diagnostics/products-under-assessment
The product was prequalified and a change request was submitted and accepted to add an intended use claim.

Malaria Rapid Diagnostic Tests: progress of the active applications in the prequalification of IVDs assessment pipeline

Product name	Product code(s)	Manufacturer name	Dossier review	Quality Management System review	Product performance evaluation	Labelling review	Application number
BIOCREDIT Malaria Ag Pf/Pv (pLDH/pLDH)	C61RHG25 and C61RHH25	RapiGen Inc	🗁	🗁	◆		PQDx 13194-160-00
BIOCREDIT Malaria Ag Pf (pLDH)	C14RHG25 and C14RHH25	RapiGen Inc	🗁	🗁	◆		PQDx 13192-160-00
Malaria P.f. Ag Rapid Test Cassette (Whole Blood)	GCMAL (pf)- 402a-1T, GCMAL (pf)-402a-25TX, and GCMAL (pf)-402a-25TD	Zhejiang Orient Gene Biotech Co., LTD	🗁				PQDx 12442-12940-00
BIOCREDIT Malaria Ag Pf (pLDH/HRPII)	C13RHG25 and C13RHH25	RapiGen Inc	🗁	🗁	◆		PQDx 13193-160-00
STANDARD Q hs-Malaria P.f/P.v Ag Test	09MAL70D	SD Biosensor, Inc.	🗁		🗁		PQDx 13200-117-00
Lariacheck Pf	302100025, 302100100, 302100025(1T), 302100005, and 302100010	Orchid Biomedical Systems_Division of Tulip Diagnostics (P) Ltd	🗁	◆	◆		PQDx 0694-024-00
ErbaQik Malaria Ag Pf/Pan	135343 and 135342	Transasia Diagnostic Pvt Ltd.	🗁				PQDx 12405-12158-00
ErbaQik Malaria Ag Pf/Pv	135346 and 135347	Transasia Diagnostic Pvt Ltd.	🗁				PQDx 12409-12158-00
MERISCREEN Malaria Pf HRP-II/pLDH Ag	RWPFDH-09, RWPFDH-01, RWPFDH-02, RWPFDH-03, RWPFDH-04, RWPFDH-05, RWPFDH-06, RWPFDH-07, RWPFDH-08, RWPFDH-10, RWPFDH-11, RWPFDH-12, RWPFDH-13, and RWPFDH-14	Meril Diagnostics Pvt. Ldt.	R	◆	🗁		PQDx 13179-074-00
STANDARD Q hs-Malaria P.f Ag Test	09MAL60D	SD Biosensor, Inc.	🗁		🗁		PQDx 13199-117-00

Why 5% threshold ?

Update of the response plan to *pfhrp2* gene deletions

Meeting report, 26 January 2023

- 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

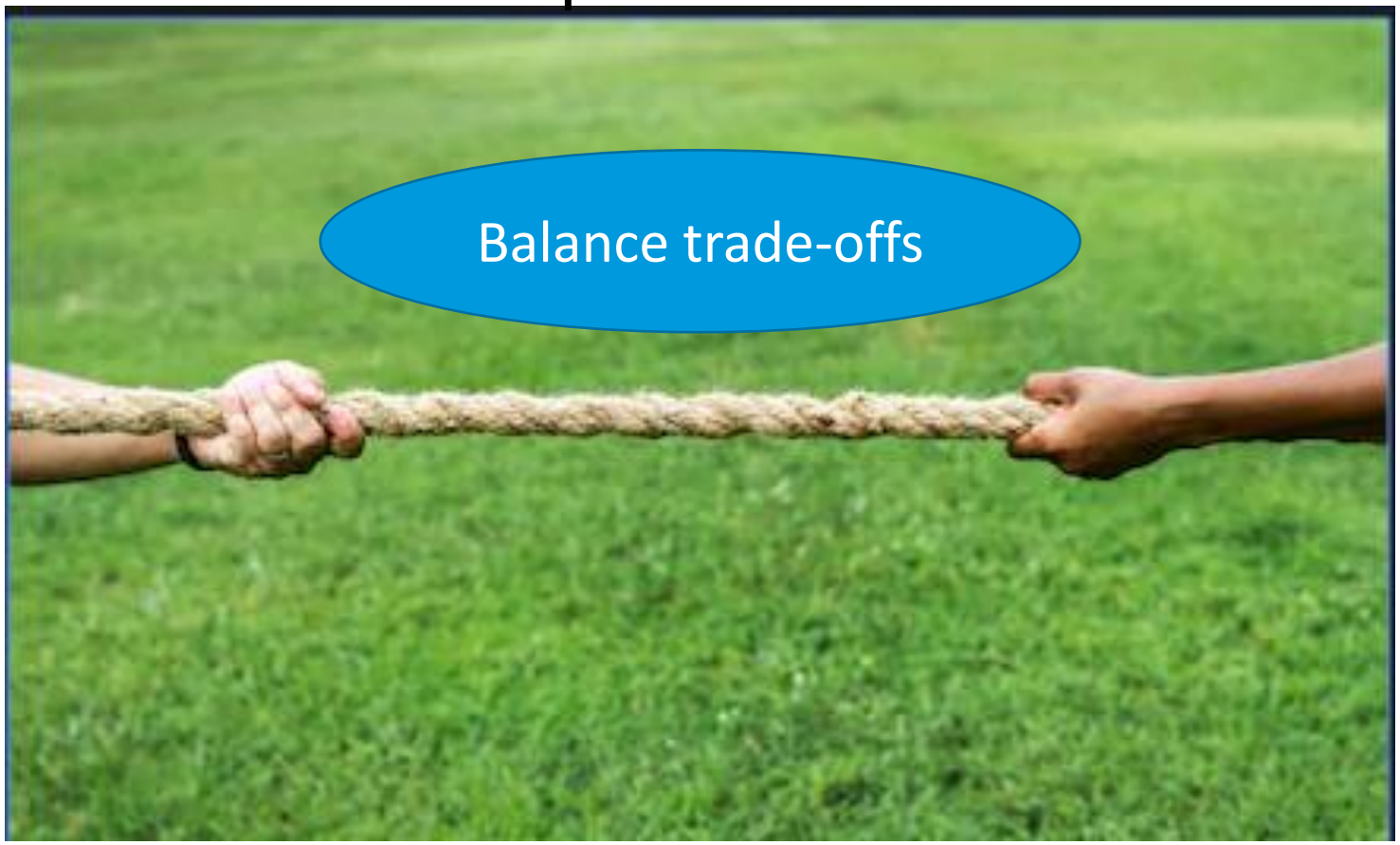
Review in 2022 – n=24 studies

Table 1. Sensitivity and specificity of HRP2-detecting RDTs relative to Pf-LDH-detecting RDTs

Microscopy as the reference standard		PCR as the reference standard		Overall	
Sensitivity %	Specificity %	Sensitivity %	Specificity %	Sensitivity %	Specificity %
4.3	-8.8	12.9	1.3	8.0	-5.3
Excluding one study in Peru with a very high prevalence (26%) of *pfhrp2* gene deletions					
5.9		-		9.0	

https://iris.who.int/bitstream/handle/10665/378767/9789240097919-eng.pdf?sequence=1

Up to 2025

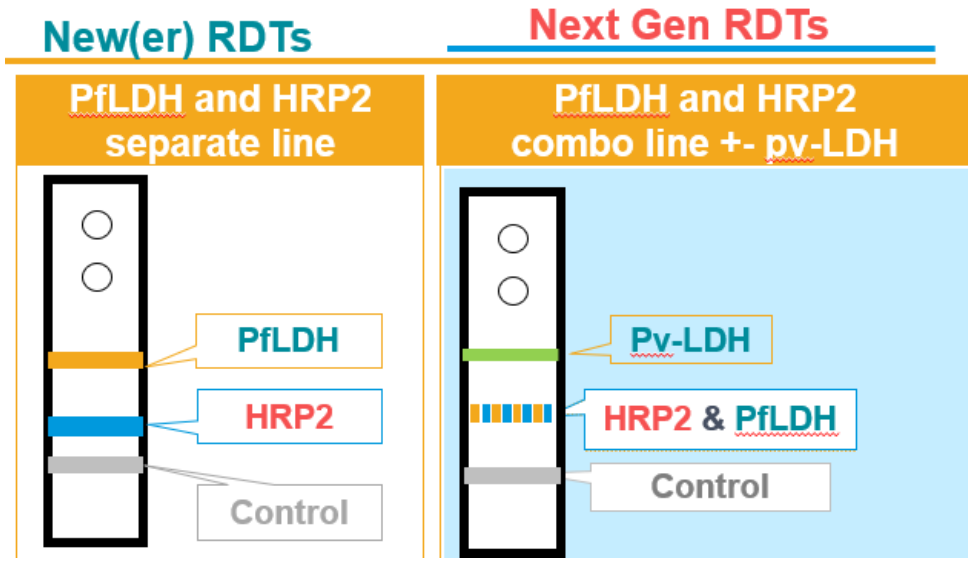


HRP2-RDT negative due to pfhrp2/3 deletions

VS.

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

Future



No trade off in performance

Keep benefit of HRP2 and more sensitive pf-LDH test lines

The trade off will be about cost : additional cost of next Gen RDT vs cost of surveillance and false negative results (health and public health impact)

Network of Reference Laboratories

Contact – cunninghamj@who.int for linkage with reference lab network member who can support molecular analysis needs

Country	Name of institute
United Kingdom of Great Britain and Northern Ireland	Malaria Reference Laboratory/Faculty of Infectious Diseases/ London School of Hygiene and Tropical Medicine
United States of America	University of North Carolina at Chapel Hill
Australia	Australian Defence Force Malaria and Infectious Disease Institute and QIMR Berghofer Medical Research Institute
United States of America	Centers for Disease Control and Prevention
Senegal	Université Cheikh Anta Diop de Dakar, and International Center for Research and Training in Applied Genomics and Health Surveillance
Peru	Universidad Peruana Cayetano Heredia
India	National Institute of Malaria Research

2025: We're expanding and evolving….

Reference Lab network has supported molecular analysis for pfhrp2/3 deletions in several countries DRC, Peru, India, Eritrea, Ethiopia, Somalia, Yemen, Kenya, Tanzania, S Sudan, Djibouti….

Working through tripartite agreements - WHO- MOH-reference laboratories

Sharing data for policy before publication

Expanding to include markers of drug resistance

World Health Organization

The spread of molecular markers of artemisinin partial resistance and diagnostic evasion in Eritrea: a retrospective molecular epidemiology study

Selam Mihreteab, Karen Anderson, Irene Molina de la Fuente, Colin J Sutherland, David Smith, Jane Cunningham, Khalid B Beshir, Qin Cheng

Lab methods for pfhrp2/3 deletions

Beshir *et al. Malaria Journal* 2022, 21:201
https://doi.org/10.1186/s12936-022-04226-2

Malaria Journal

REVIEW Open Access

Screening strategies and laboratory assays to support *Plasmodium falciparum* histidine-rich protein deletion surveillance: where we are and what is needed

Khalid B. Beshir[1†], Jonathan B. Parr[2†], Jane Cunningham[3], Qin Cheng[4,5] and Eric Rogier[6*]

- Resources including protocols on MESA platform

Advantages	Limitations
Conventional PCR	
• Can be performed in most molecular laboratories and is widely used in many countries • Can detect deletions of both exons • Can identify deletions of flanking genes	• Time consuming at > 2 hours per reaction and qualitative method requiring visualization of PCR products on agarose gels • Requires multiple different PCR reactions; > 6 reactions per sample for control and *pfhrp2/3* genes and high volume of DNA needed • Nested PCR and higher chances of contamination • Cannot detect gene deletions when gene-deleted and non-deleted parasites are mixed in the same sample
Multiplex real-rime PCR	
• Streamlined workflow with short turnaround time • Quantitative read-out • Can detect mixed infections with gene-deleted and non-deleted strains • Different target genes are multiplexed in a single reaction, requiring less volume of DNA	• Requires multichannel real-time PCR machine • Training is required for proper interpretation of results • Careful optimization required for individual laboratories • May not detect some partial gene deletions involving one exon, as most assays target one exon only
Digital droplet PCR	
• Higher confidence deletion calls than with other molecular methods • Can clearly detect mixed infections with gene-deleted and non-deleted strains	• Requires specialized equipment that is not widely available • Requires advanced laboratory and analysis expertise and training • More expensive than conventional approaches
Sequencing approaches	
• The exact chromosomal location and fragment size of deletions, as well as sequence changes that change codons or affect HRP2/3 expressions, can be mapped • Amplicon-based and whole-genome-based next-generation sequencing methods can be used for parasite population structure and relatedness • Complexity of infection and evolutionary analysis are possible when appropriately sampled data are generated	• More suitable for research studies than for routine programmatic use, as it is expensive and requires advanced laboratory infrastructure and bioinformatics analysis support • Current approaches are not well suited for initial deletion identification, especially in lower parasite density samples

World Health Organization

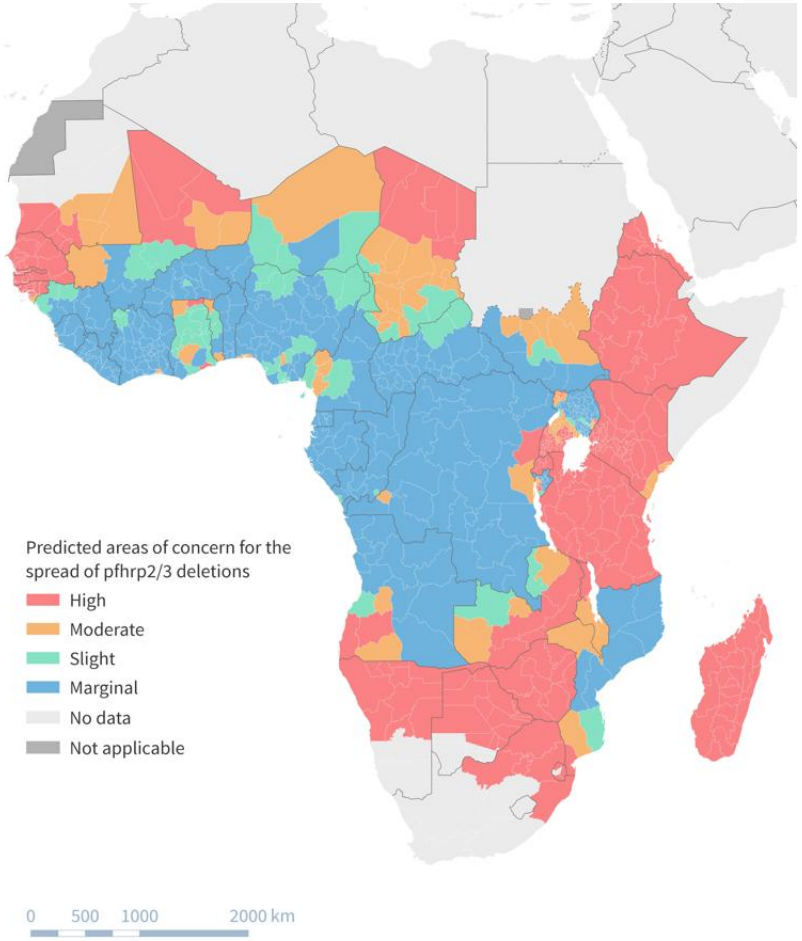
Future planning

Modelling predicts that *hrp2/3* gene deletions at clinically relevant levels will continue to emerge and spread throughout Africa

Areas of lower burden are most at risk[1]

Global risk of selection and spread of *Plasmodium falciparum* histidine-rich protein 2 and 3 gene deletion

Oliver J. Watson[1], Thu Nguyen-Anh Tran[2], Robert J Zupko[2], Tasmin Symons[3], Reb Thomson[4], Theodoor Visser[5], Susan Rumisha[3], Paulina A Dzianach[3], Nicholas Hatha Isaac Kim[7,8], Jonathan J. Juliano[9,10,11], Jeffrey A. Bailey[7,8,12], Hannah Slater[13], Lucy O Peter Gething[3,14], Azra Ghani[1], Maciej F Boni[2,15], Jonathan B. Parr[10,11], Jane Cunninghan



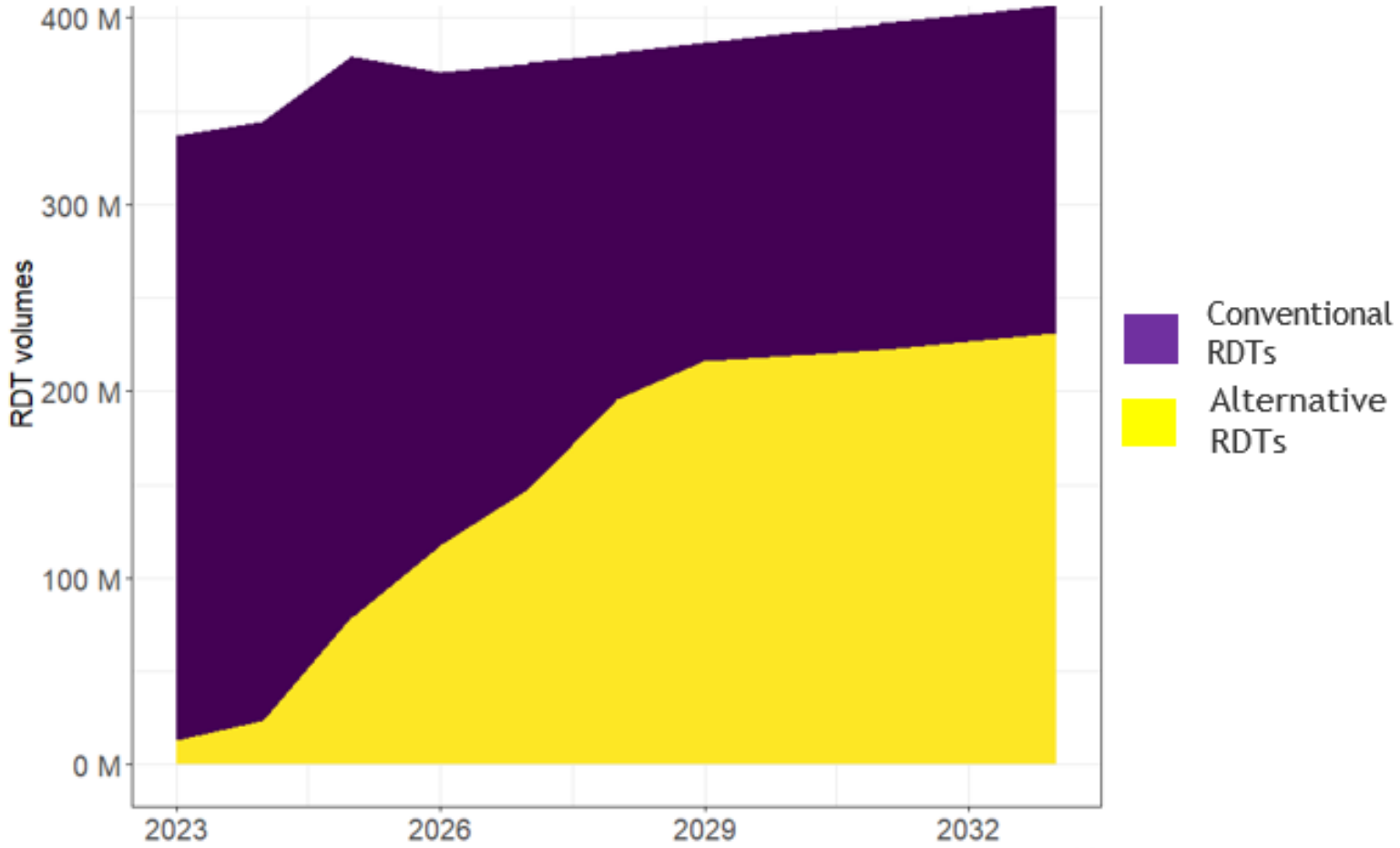
Drivers of selection[2]

1. Transmission intensity
2. Treatment coverage
3. Incidence of non-malarial fevers
4. Proportion of clinical cases that are tested using microscopy
5. Adherence to diagnostic test outcomes for treatment
6. Size of the private drug market and diagnostic practices of private market
7. Fitness costs associated with *pfhrp2/3* deletions
8. Cross-reactivity of HRP2-based RDTs with HRP3
9. Frequency of *pfhrp3* deletions
10. Do *pfhrp2* deletions occur independently to *pfhrp3* deletions

1 - https://pmc.ncbi.nlm.nih.gov/articles/PMC10615018/pdf/nihpp-2023.10.21.23297352v3.pdf
2 - https://worldhealthorg.shinyapps.io/DeletionRiskExplorer/

The demand for non-exclusive HRP2 based *P. falciparum* RDTs will grow

- By 2032 up to half of the market could be made up of new/alternative RDTs
- Wake-up call for manufacturers and procurers
- Innovative, evaluation
- Market shaping



World Health Organization

Mapping the distribution and frequency of *pfhrp2/3* deletion mutants with harmonized protocols – 2020; corrigendum 2023, Dec 2024

World Health Organization

Core surveillance approach for efficiently detecting pfhrp2/3 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

World Health Organization

Key Revisions

- All Pf cases /denominator – all pf-LDH+ or microscopy +

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

- Using lab-based immunoassay (ELISA, chemiluminescent assay and multiplex bead-based assay) – screen for HRP2, LDH, instead of 2 RDTs
  - High throughput, field worker independent but routine RDTs results still required; cannot distinguish pfhrp2 and pfhrp3; commercial and non-commercial options; US CDC supported several surveys
- Range of molecular methods and sequencing to confirm/exclude deletions
  - Participation in WHO NAAT EQA scheme
    - https://www.who.int/teams/global-malaria-programme/case-management/diagnosis/nucleic-acid-amplification-based-diagnostics/faq-nucleic-acid-amplification-tests

World Health Organization

Key Revisions: Sampling approach – based on a DOMAIN

- Original protocol – based on the true prevalence of *pfhrp2* deletions being between 3-8%
- 10 health facilities per domain (e.g. province, region)
- enrol 37 *P.falciparum* patients in each site = 370 in total per domain
- in order to reach 80% power
- 2 tailed test to detect prevalence above or below the threshold of 5%

Sample sizes for determining if the true *pfhrp2* deletion prevalence is above or below the 5% threshold at the survey domain (province) level

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
5.0	
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

Error identified - design effect not included in sampling and other methodological concerns raise

Corrigendum published and new sampling approach explored

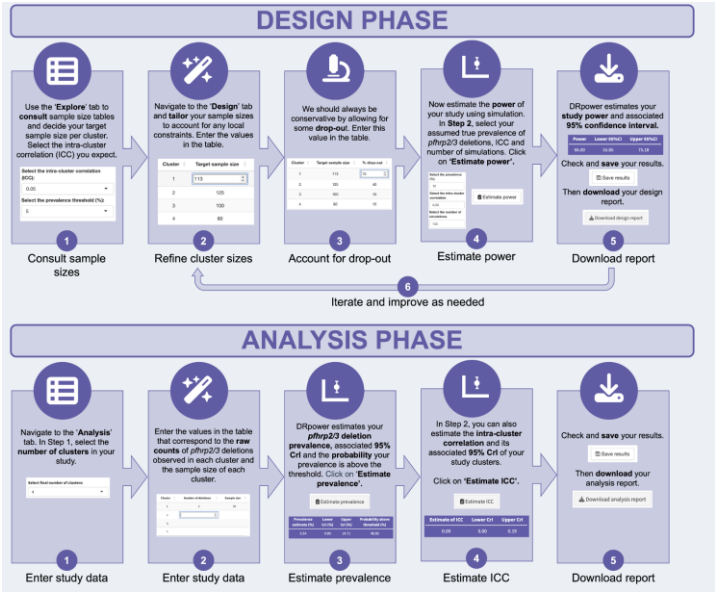
World Health Organization

Key Revisions: Sampling approach (Dec 2024)

- uses a Bayesian statistical approach while allowing for correlations within clusters
- higher power
- allows integration of information from other *pfhrp2/3* studies
- one tailed test to identify areas that are *above* the 5% threshold
- the number of clusters (health facilities) can be selected based on practicalities of the study location
- the higher the number of clusters included, the lower the overall sample size
- sample size has not changed significantly from original protocol - 30 instead of 37 *P.falciparum* cases per health facility/cluster
- Each design and analysis phase is per domain

Table 2. Sample size required to achieve 80% power using the proposed analysis

Number of clusters (health facilities)	Sample size per cluster	Total sample size
1	--	--
2	--	--
3	--	--
4	--	--
5	496	2 480
6	113	678
7	68	476
8	51	408
9	37	333
10	30	300



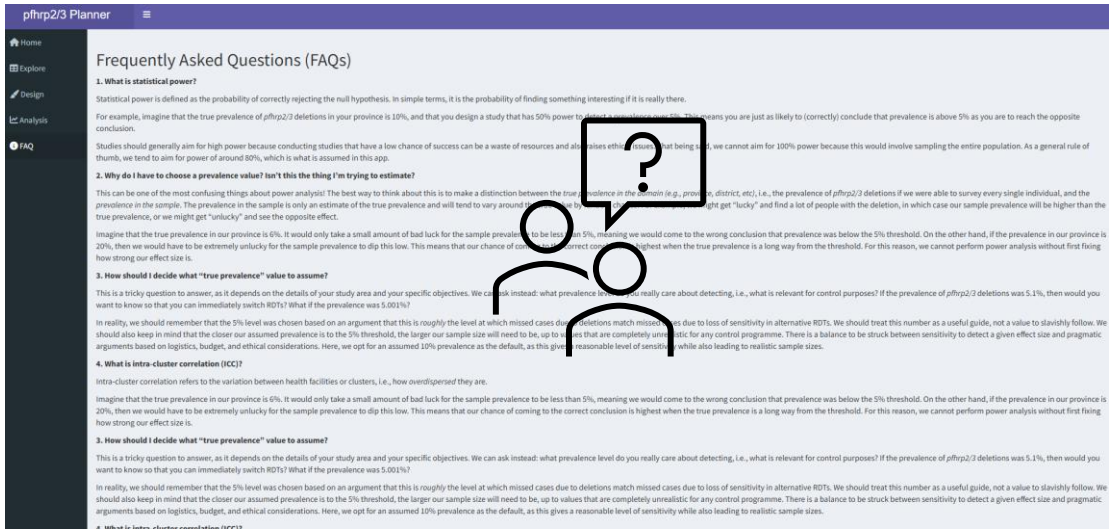
https://shiny.dide.ic.ac.uk/DRpower-app/

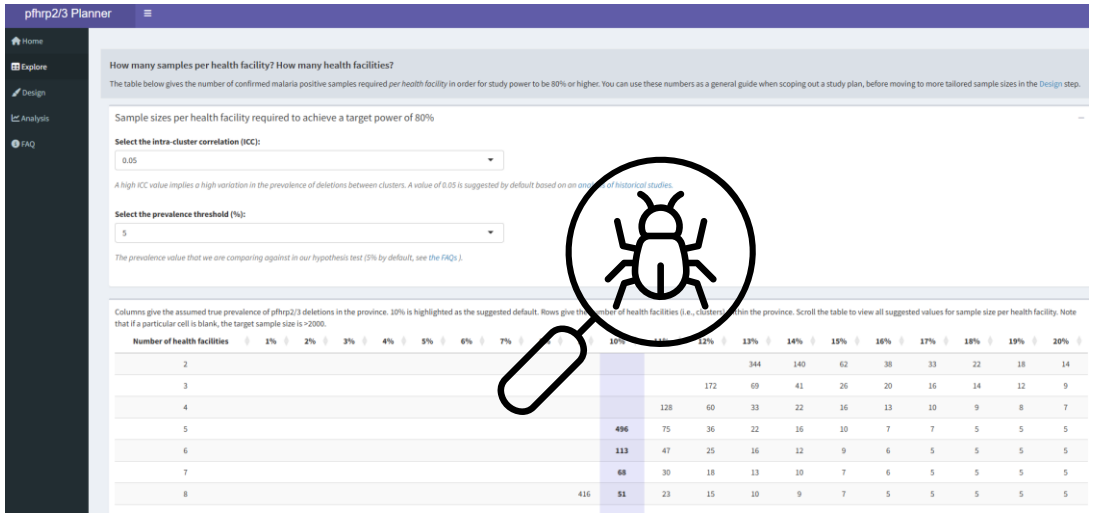
World Health Organization

HRP2 &3 gene deletion survey-20241108_160358-Meeting Recording.mp4

Pfhrp2 planner app

- The app is very flexible
  - use default settings or customize based on your site characteristics
  - It will automatically calculate the power based on changes in samples size or number of clusters/health facilities
  - It accounts for estimated drop out rates
  - If you need to drop sites during enrolment, the app can generate new samples sizes for the remaining health facilities
- If using old protocol with different sample sizes (37 per domain) – you have most likely collected sufficient samples to make 'powered' conclusions but you can enter the data into the app and find out.
- Always include domains that span all malaria transmission zones in the country - pfhrp2 deletions most likely to emerge in low transmission areas
- For more advanced analysis or further understanding visit: https://mrc-ide.github.io/DRpower/ and also the FAQ





Thank you

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Qin Cheng, Australian Defence Force Malaria & Infectious Disease Institute Laboratory
Venkatachalam Udhayakumar, Former US CDC
Spike Nowak, PATH

Credit: WHO/F.Combrink

World Health Organization

Ministry of Health

Kenya National Longitudinal Surveillance of *pfhrp2/3* deletions and biobanking

Presenters: Regina Kandie & Dr. Hosea Akala

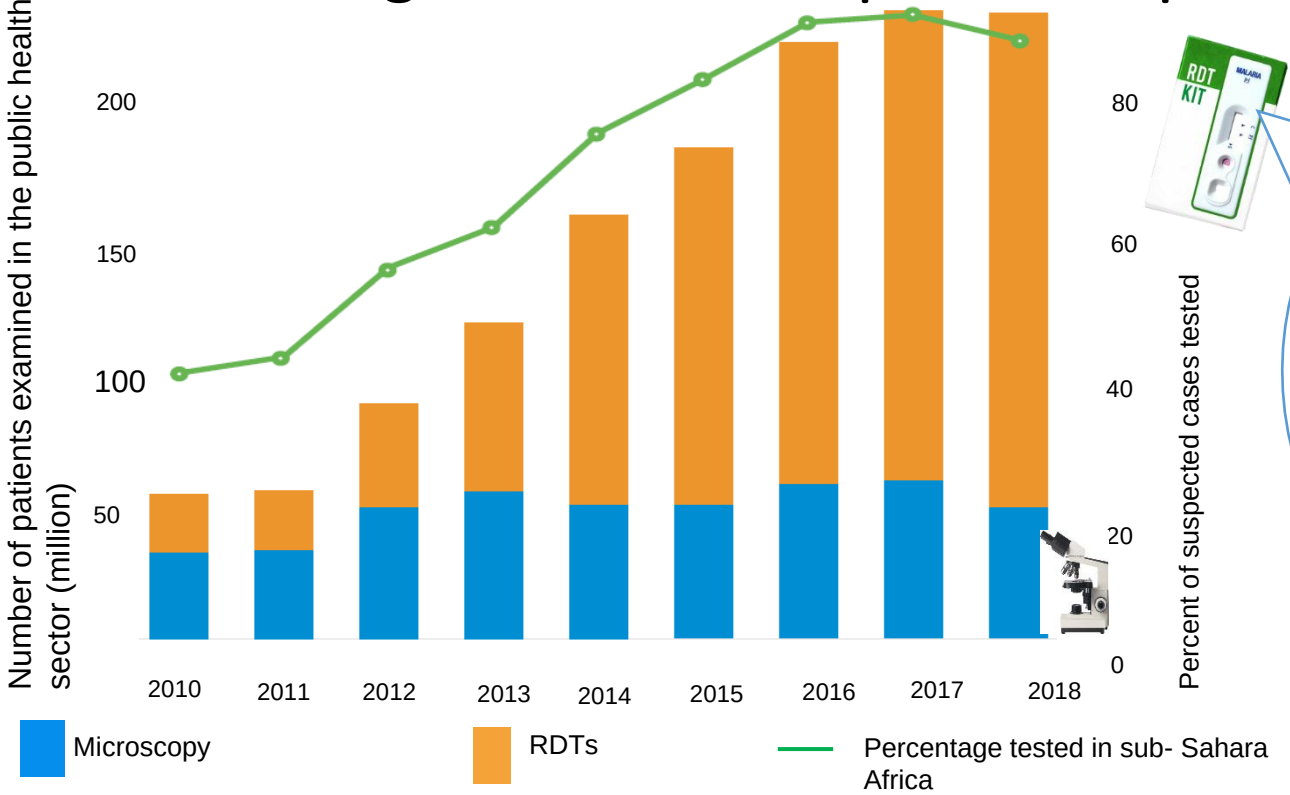
3rd April,2025

Outline

- Back ground
- Objective
- Method
- Results
- Challenges
- Learnt lesson

Introduction _1 of 2

Increasing use of RDTs in public hospitals between 2010 to 2018



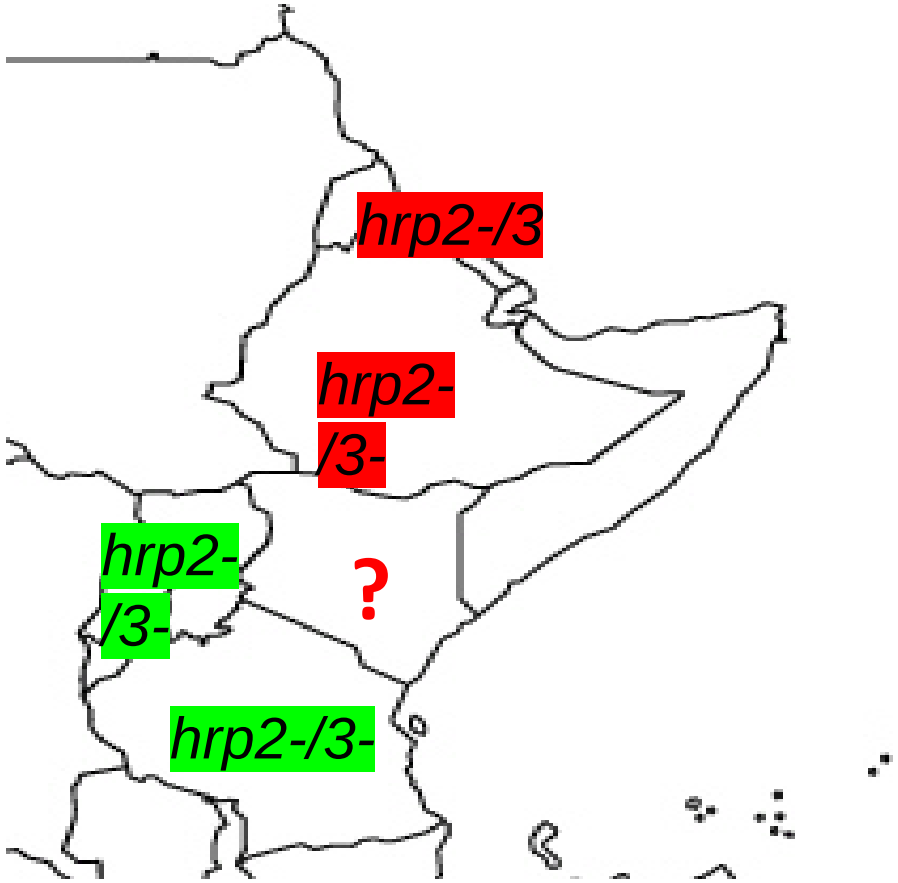
Results affected by:

- Parasite density
- Storage
- Handling of the kit
- Interpretation
- Cross-reaction with Pfhrp3
- **Lack of targeted antigen**

WHO Malaria Report 2019

Introduction_2 of 2

- Rapid Diagnostic Tests (RDTs) & malaria microscopy are the cornerstone of malaria management in clinical settings
- Persistent malaria antigen (histidine-rich protein 2) after successful treatment, leads to false positive results
- Increasing numbers of false negative results reported from malaria endemic regions due to Pfhrp2/3 gene deletions



Regional & Nationwide Diagnostic resistance

Specific objectives

1. Establish the prevalence of suspected Pfhrp2/Pfhrp3 gene deletions among symptomatic falciparum patients attending public health facilities.

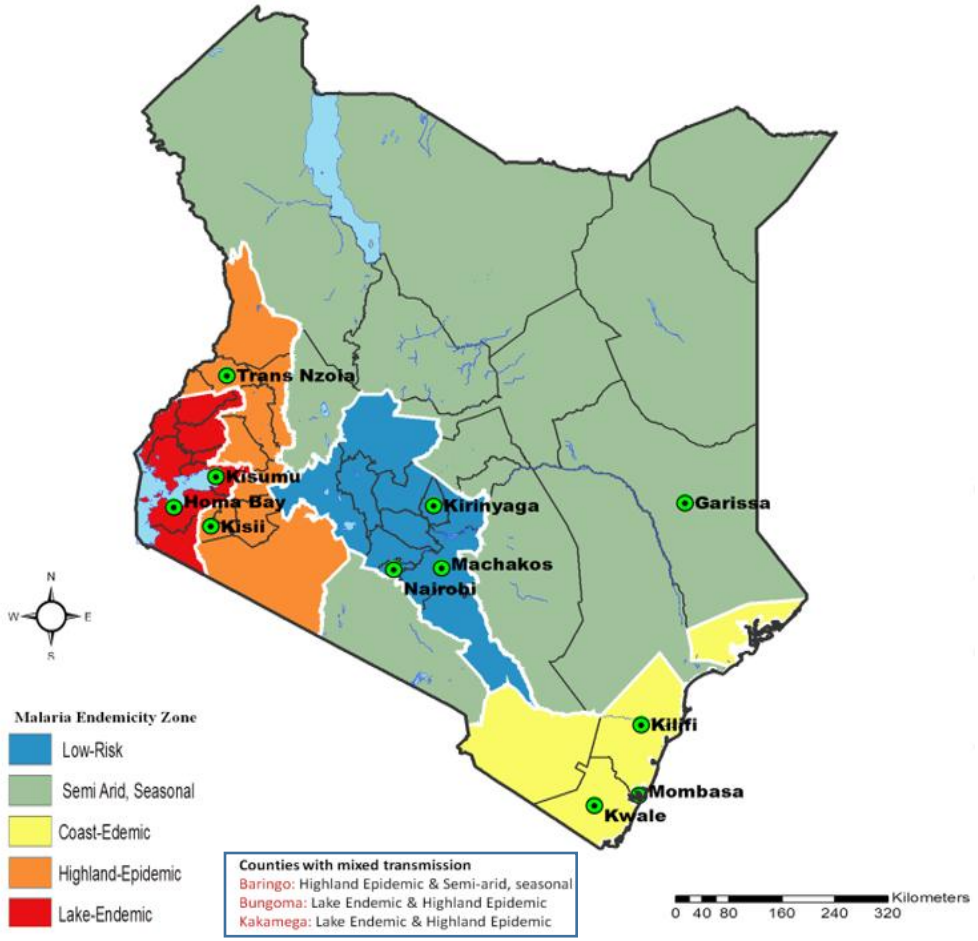
2. Detect the parasite density and frequency of Pfhrp2/3 gene deletions in that cohort.

3. Determine the predictive value of suspected false-negative Pfhrp2 RDT results for pfhrp2/3 gene deletions in different settings.

4.Identify regions in which the prevalence of Pfhrp2/3 gene deletions causing false negative *P. falciparum* RDTs is at or above 5%, warranting a change in RDTs.

Study Design and Methodology

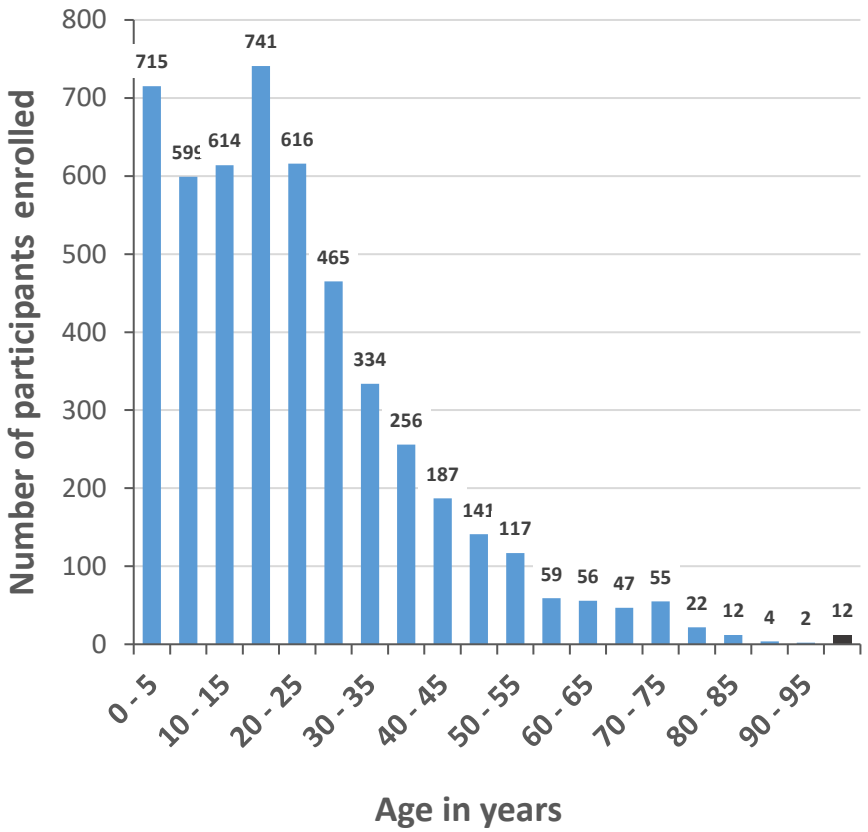
Study population	Suspected malaria cases seeking care at public health facilities	With non-representative or sporadic reports of Pfhrp2/3 deletions in the country
Anyone 6 months and above	10 Counties	**Laboratory assays** • RDT • Extract DNA • Long-term storage



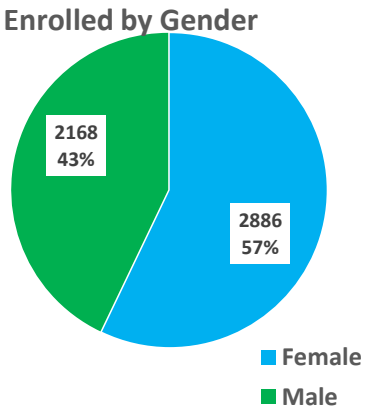
Results_1 of 4

Enrolled Participants N=5,054

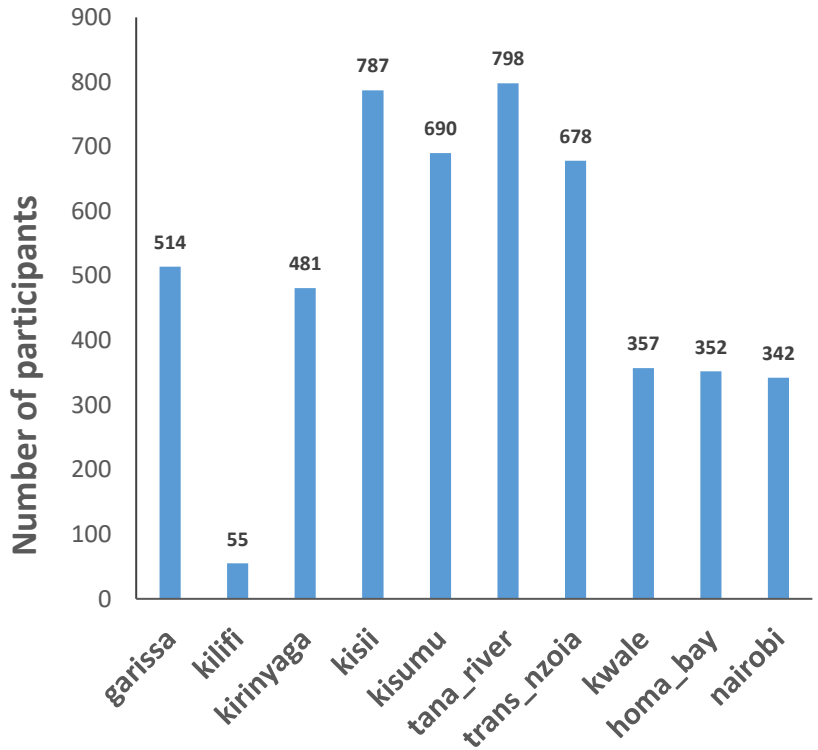
Participants Age



Enrolled by Gender



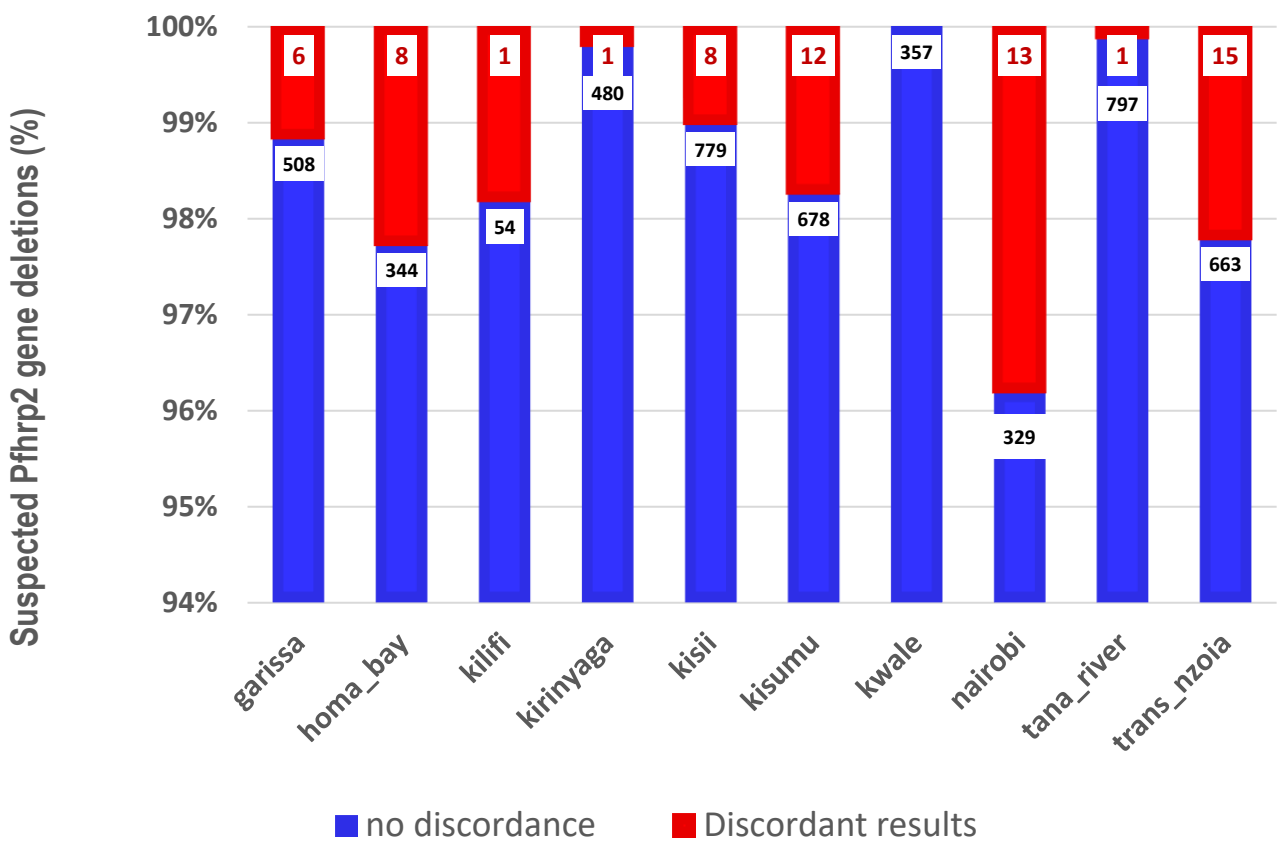
Number per County

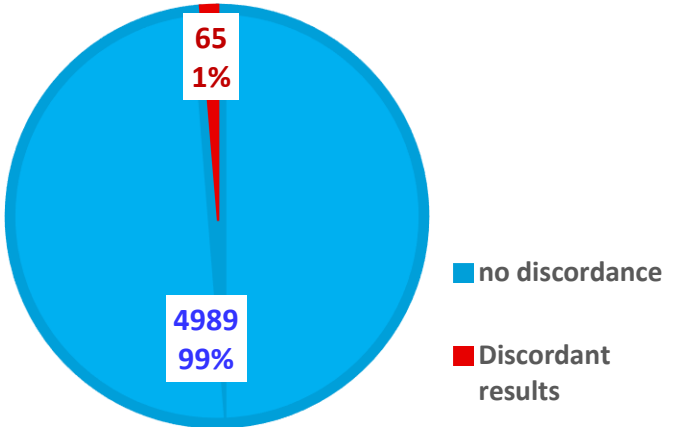


Positive by at least one test kit = 2,296(45.3%)

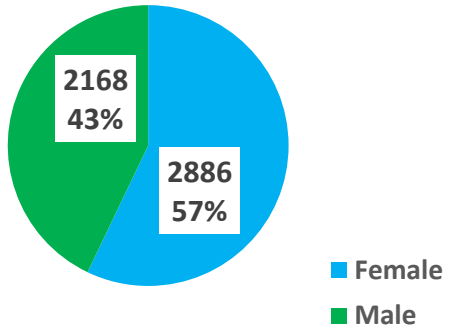
Results_2 of 4

Suspected Pfhrp2 gene deletions per county



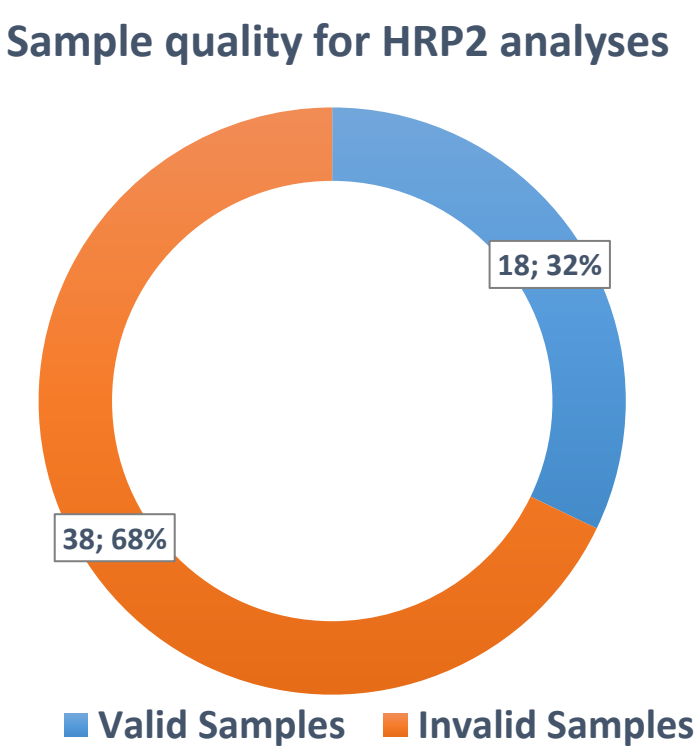


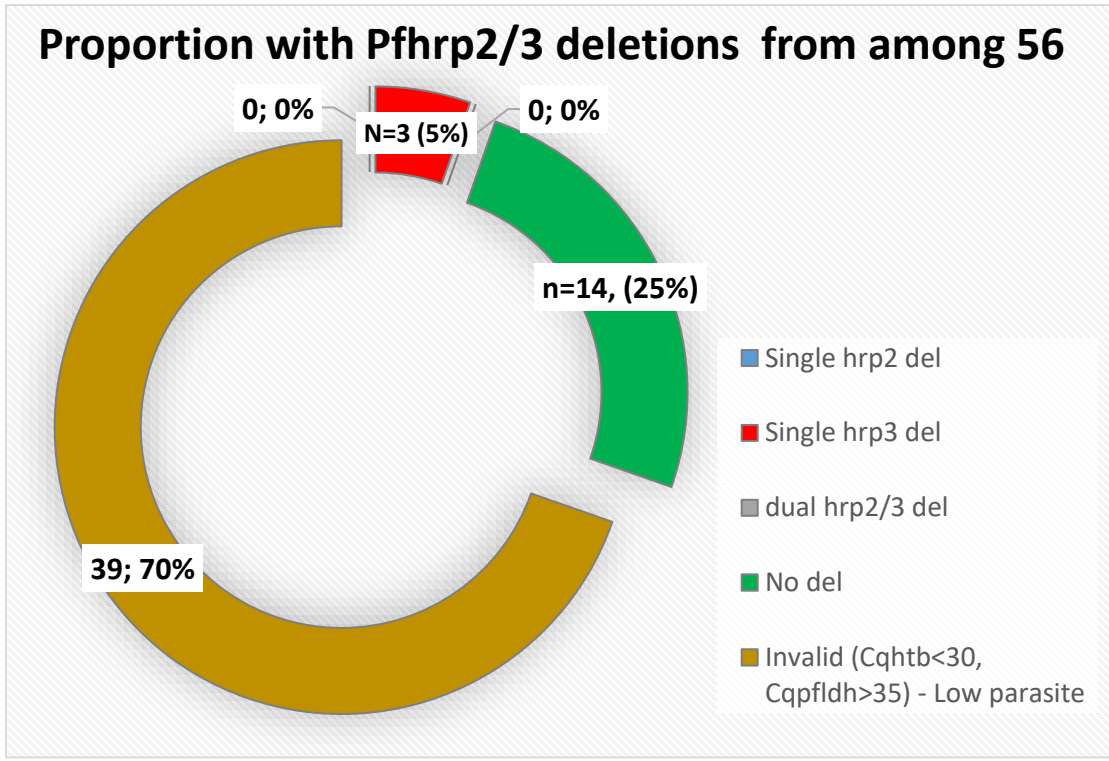
Distribution of Enrolled by Gender



Results _3 of 4

Molecular analyses of samples suspected Pfhrp2/3 deletions

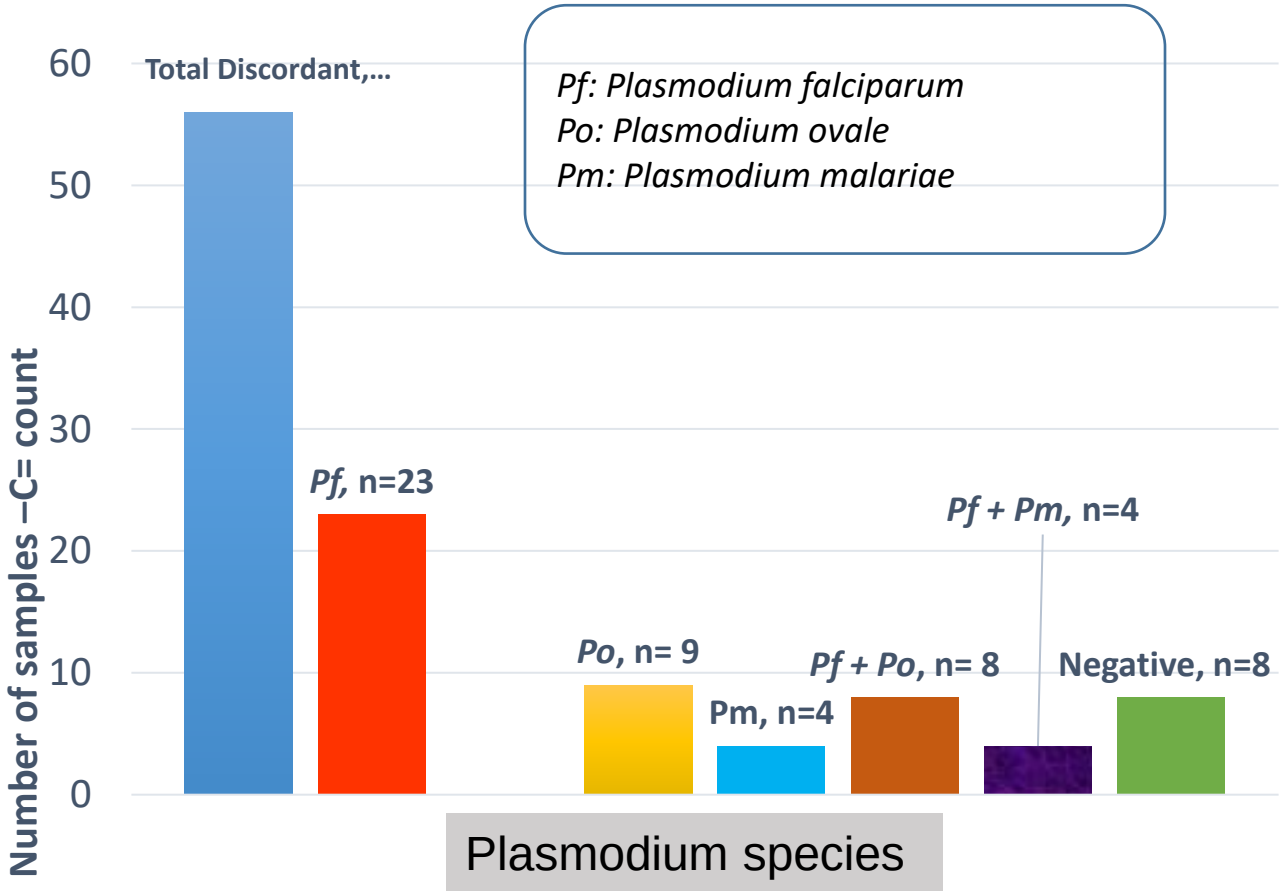




56/65 suspected Pfhrp2 deletions samples sent for analysis

Results_4 of 4

Species composition of samples with discordant Pfhrp2 diagnosis results



- A total of 48 of the 56 samples with discordant results successfully amplified for species composition
- Non-falciparum infections were detected in 25/48 (45%) of the samples with discordant Pfhrp2 results
  - Representing 25/2,296 (1%) of the infections
- The 23 *Pf* missed out by Pfhrp2 band and reactive by *Pf-p*LDH appear to suggest sensitivity of the later
-

Discussion and policy implications

- The study shows a < 1% prevalence of Pfhrp2/3 gene deletions causing false negative diagnostic results among symptomatic individuals in Kenya
- This prevalence, within the <5% categorizes the test as accurate for continued use in Kenya for testing symptomatic infections
- At this test threshold, the WHO recommends repeat survey every two years
- Non-falciparum parasitemia contribute to false negative Pfhrp2/3 gene results
- Capacity Building: Training programs should be updated to include awareness of Pfhrp2/3 gene deletions

Implementation Challenges in Pfhrp2/3 gene Deletions Studies

1. Funding Constraints

- Limited financial resources for Pfhrp2/3 deletions studies.

2. Delayed Molecular Analysis

- Slow processing and interpretation of results.

3. Seasonal Data Collection Issues

- Variations affect sample availability.

4. Extended Sample Collection Period

- Took longer than planned.

5. Sample Contamination Risk

- Affects data reliability.

6. Logistics Challenges

- Difficulties in transporting and storing samples in remote areas.

Learnt lesson

. Proper Sample Collection and Storage

- Future studies must prioritize proper handling and storage to ensure data integrity.

2. Ensuring Consistent Funding

- Sustainable funding mechanisms are needed for continuous research and surveillance.

3. Strengthening Microscopy Methods

- Particularly important where Pfhrp2/3 deletions are prevalent to ensure accurate diagnosis.

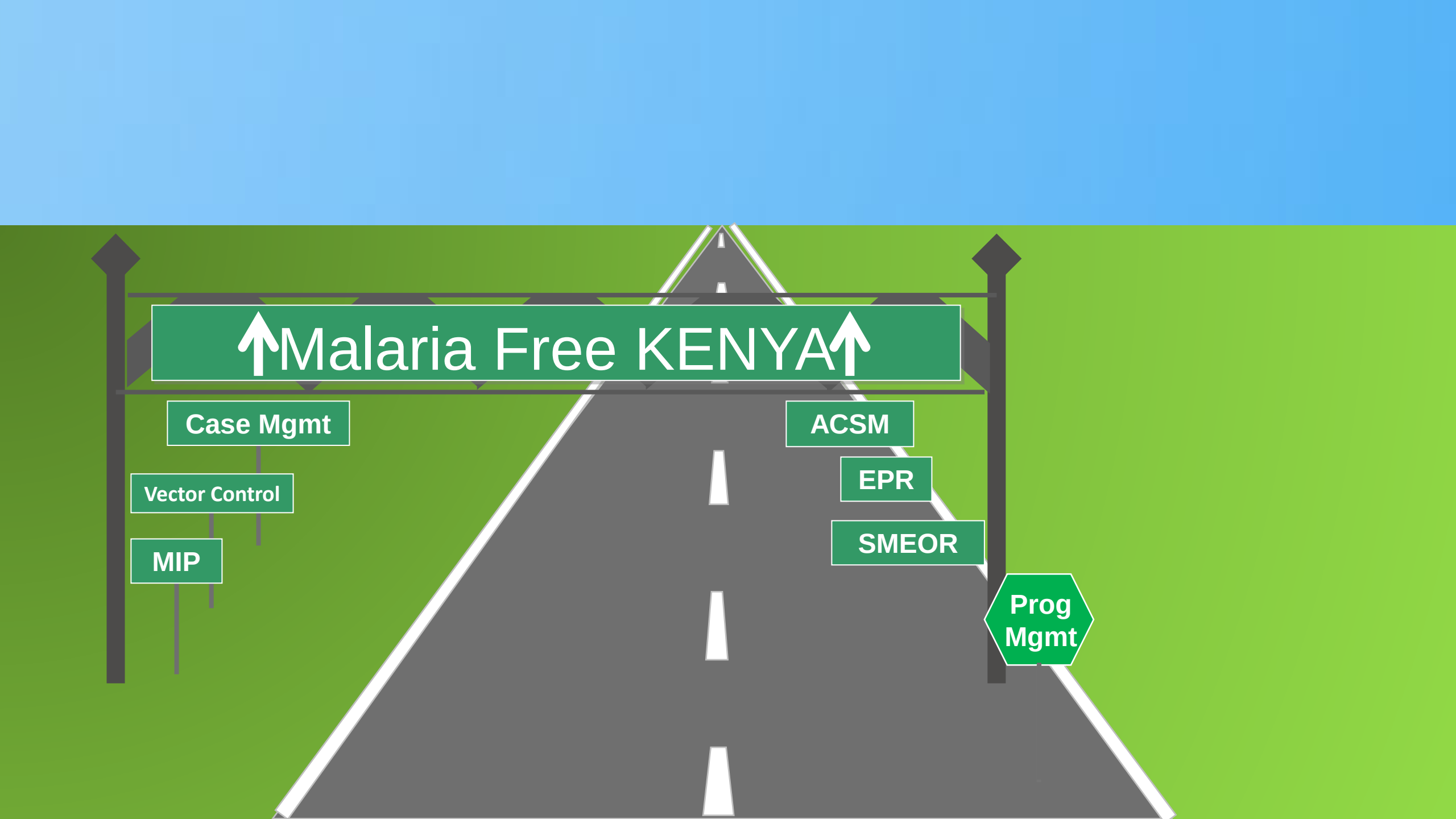
4. Enhancing Laboratory-Based Diagnostics and Quality Control

- Improved protocols and training are essential for maintaining diagnostic accuracy.

5. Continuous Monitoring

Acknowledgments

- Global fund
- WHO
- NMCP
- KEMRI
- KMTC
- NPHI
- Counties
- Australian defence force malaria and infectious disease institute, the WHO reference laboratory


Malaria Free KENYA
Case Mgmt
Vector Control
MIP
ACSM
EPR
SMEOR
Prog Mgmt

Ministry of Health
Republic of South Sudan

Prevalence of *pfhrp2/3* deletions in South Sudan: Results of a 10-site national survey

Matthew Coldiron, MSF

MESA Forum Webinar

3 April 2025

Désiré Ndisabiye, Olivier Denis, Pascale Chaillet, Letizia Di Stefano, Erwan Piriou, Constantino Doggale, Jane Cunningham, Valérie Briand, Qin Cheng, Matthew E Coldiron

Malaria in South Sudan

- 2022[1]:
  - Population: 10.9M
  - 9.9M suspected cases
  - 2.5M confirmed cases
  - 4429 reported deaths
- Differential malaria transmission:
  - North: highly seasonal
  - South: holoendemic
- Emergence of HRP2/3 deletions in neighboring Ethiopia and artemisinin resistance in neighboring northern Uganda

[1] World Malaria Report 2023

pfhrp2/3 deletions in South Sudan

- Two small-scale single-site studies (not designed to evaluate *pfhrp2/3* deletions) looked for deletions post-hoc
  - Aweil (north, seasonal): 0.6% of febrile children had *pfhrp2/3* deletions[1]
  - Yei (south, holoendemic): 7.6% of children with postive pLDH RDT had *pfhrp2/3* deletions[2]
- One study among returning travelers to Australia showed 4 of 17 had *pfhrp2/3* deletions[3]
- MSF has a large operational footprint around country

[1] Malar J. 2022;21:261
[2] Emerg Infect Dis. 2023;29:154–9
[3] Emerg Infect Dis. 2021;27:471–9

Study methods (1)

- Based on WHO standard protocol for monitoring of *pfhrp2/3* deletions

- 10 Médecins Sans Frontières (MSF)-supported health facilities around South Sudan

Study methods (2)

- Inclusion criteria
  - Suspected malaria of target age group at given MSF-supported facility
    - Fever (38°C axillary) or history of fever in preceding 48 hours
    - Generally either <5 or <15 years old
  - Written consent (plus assent from >7 years old)

- Exclusion criteria
  - Signs of severity (referred)
  - Previous enrolment in study

Study methods (3)

- Brief questionnaire
- pLDH (Rapigen Pf-pLDH RDT) and HRP2 RDTs (differed by sites) performed in parallel
- Dried blood spots from capillary blood collected directly on filter paper

- Molecular characterization at ADFMIDI, Brisbane
  - WHO CC for malaria and member of WHO *pfhrp2* Deletion Detection Network
  - Multiplex quantitative PCR which amplifies *pfhrp2, pfhrp3, pfldh* and human tubulin genes
  - All pLDH or HRP2 positive samples tested by PCR; 20% of HRP2-/pLDH- samples as well
  - Double deletions (and subset of non-deleted samples) confirmed by ELISA
  - K13 gene amplified from appx 50% of positives at each site

Sample size

- Based on updated WHO protocol (Bayesian)

- Assumptions:
  - Only 8 of 10 selected sites would be able to complete data collection
    - Enrolling 149 pLDH positives/site would allow for exclusion of 5% threshold recommended for switch from HRP2 to pLDH RDT
    - If all 10 sites successful, would only need 80 pLDH positives/site to exclude the threshold
  - Appx 60% RDT-positivity → target enrolling 200 suspected cases/site
    - Due to remote nature of sites, could not do real-time monitoring of positivity

Findings (1)

- 1842 participants enrolled between January 22 – March 27, 2024
  - Median age 3 years (1-8)
  - 9% reported malaria treatment in 2 weeks prior to enrolment

Site	Number of participants (N)	Resident of county of study site, %	Arrived in South Sudan within 30 days prior to recruitment, %
Lankien	190	93	3
Malakal	200	60	37
Aweil	185	97	1
Kajo Keji	188	99	0
Gomgoi	200	47	5
Bentiu	196	89	2
Ulang	201	96	3
Old Fangak	127	97	2
Maruwa	199	93	5
Yei	156	100	2
Total	1842	86	6

Findings (2) – RDT results

	HRP2		pLDH		pLDH positive and HRP2 negative		
Site	Valid tests	% positive	Valid tests	% positive	N pLDH positive	n HRP2 negative	%
Lankien	190	58	190	50	95	2	2.1
Malakal	200	35	200	27	53	2	3.8
Aweil	185	44	183	32	59	0	0
Kajo Keji	188	51	188	48	90	3	3.3
Gomgoi	200	31	200	22	44	0	0
Bentiu	196	13	195	12	23	1	4.3
Ulang	201	40	201	29	59	3	5.1
Old Fangak	127	65	127	56	71	1	1.4
Maruwa	199	21	199	19	37	2	5.4
Yei	156	51	156	34	53	1	1.9
Total	**1842**	**40**	**1839**	**32**	**584**	**15**	**2.6**

Findings (2) – RDT results

	HRP2		pLDH		pLDH positive and HRP2 negative		
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Gomgoi	200	31	200	22	44	0	0
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Total	**1842**	**40**	**1839**	**32**	**584**	**14**	**2.6**

Findings (3) – Characterization of RDT-discordant samples

- 14 HRP2- / pLDH + samples
  - 4 negative by PCR
  - 3 positive for *P. falciparum* – (2 with *pfhrp2/3* double deletions)
  - 4 positive for *P. ovale*
  - 3 positive for *P. malariae*

- Prevalence of *pfhrp2/3* deletions causing false-negative HRP2-RDT:
  - **2/579 (0.35%, 95%CI [0.04-1.24])**
  - No individual site approached the 5% threshold
    - 1 double deletion at Lankien (1.05%), 1 double deletion at Ulang (1.85%)

K13 mutations

- C469Y:
  - 0.8% (3/381; 95%CI [0.04-1.26])
  - 2 in Kajo Keji (3.5% of positives), 1 in Yei (2.9% of positives)
    - Both in south of country near border with northern Uganda

- R622I:
  - 0.5% (2/381; 95%CI [0.06-1.88])
  - 1 in Malakal (2.8%) and 1 in Gomgoi (2.9%)
    - Both in north of country near border with Sudan

Discussion and conclusions

- *pfhrp2/3* deletions leading to false-negative HRP2 RDT were rare
  - Do not meet threshold for switch to pLDH RDT
- Decent geographic reach of survey, nonetheless not a nationally-representative sample
  - Continued follow-up needed
- Which pLDH RDT to use in future surveillance activities?

Challenges and Lessons Learned

- Administrative aspects of establishing a partnership
- Ensuring supervision in remote and difficult-to-access areas
- Implementation delays led to missing seasonal peaks (and hence under-enrollment at some sites)
- Operational compromises made for efficiency's sake – was it worth it?
  - Limited number of pLDH tests available
- Opportunistic monitoring of k13 mutations

Thanks

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Désiré Ndisabiye
Olivier Denis
Pascale Chaillet
Letizia Di Stefano
Erwan Piriou
Alain Ngoko

Ministry of Health of South Sudan
Constantino Doggale

WHO
Jane Cunningham
Andrea Bosman

Epicentre
Valérie Briand
Céline Langendorf

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

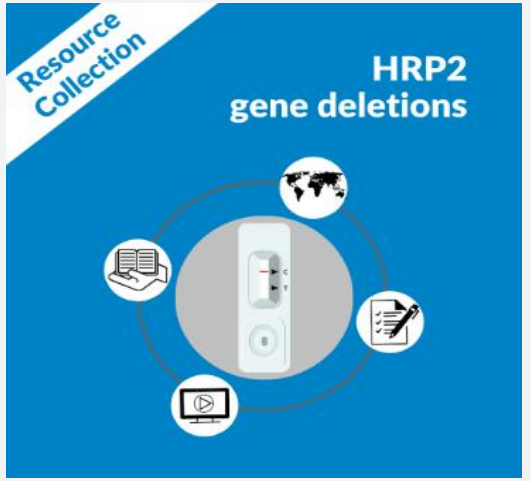
MESA FORUM
@MESAmalaria

Community of Practice
pfhrp2/3 gene deletions

Mobilizing and providing peer and technical support

→ CoP **information** page
→ CoP **registration** online form

MESA Resource Collections:



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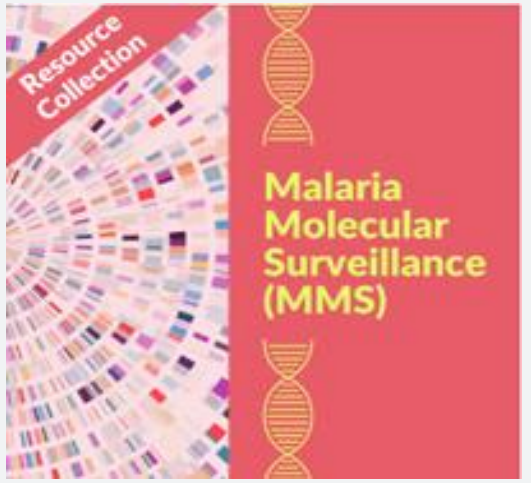

→ link

MESA Resource Collection

Responding to the threat of *pfhrp2/3* deletions

A curated list of valuable resources designed to assist the malaria community in addressing the challenges posed by this urgent threat to malaria diagnosis

→ link

WHO materials:
- Response plan & guidance
- Template protocols
- Map & dashboard

Apps:
- *Pfhrp2/3* Planner
- Deletion Risk Explorer

- CoP updates
- Registration form

- Prequalified/ eligible RDTs
- Procurement guidance

- Videos
- Interviews
- Articles

- MESA Forums
- Symposiums
- Webinars

Literature reviews:
- Sampling
- Laboratory methods
- Meta-analyses