MESA

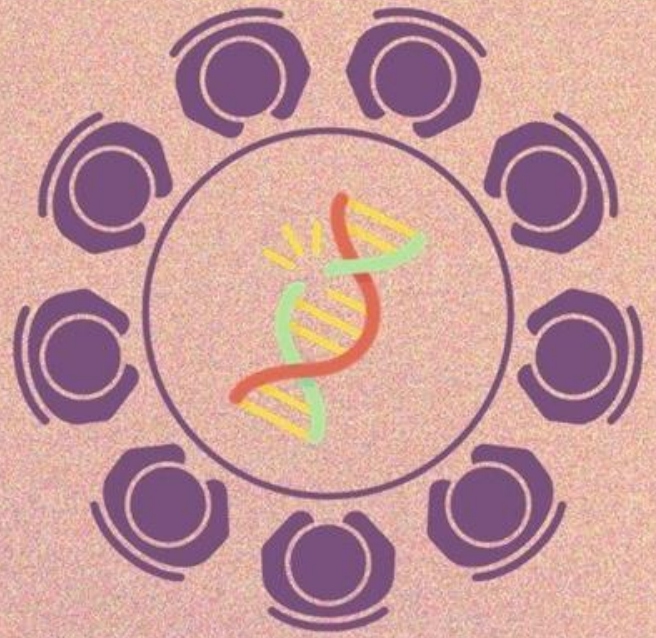
MESA FORUM

VIRTUAL | 7 JUNE, 2 PM CET

Community of Practice

pfhrp2/3 gene deletions

Mobilizing and providing peer and technical support



#MESAForum

@MESAmalaria

@MESA Malaria

Presentation of the Community of Practice

Deus Ishengoma (NIMR, Tanzania)

Implementing a national survey: Lessons from Uganda

Bosco Agaba (NMCP, Uganda)

Network of reference laboratories for surveillance activities

Qin Cheng (ADFMIDI, Australia)

Methods available to detect *pfhrp2/3*-deleted parasites

Khalid Beshir (LSHTM, UK)

Community of Practice (CoP) on *pfhrp2/3* gene deletions

– *Mobilising and providing peer and technical support* –

Overview and current status of the CoP

Deus Ishengoma

(Chair)

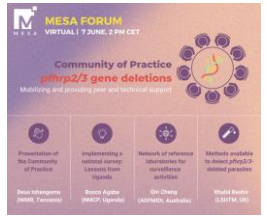
NIMR, Tanzania

7th June 2023

WHY: rationale for the creation of this CoP

MESA Resource Compilation: protocols, guidance documents, etc.

First CoP event: **MESA Forum**



May 2021

- Countries willing to set up surveys, but lack experience and/or technical resources to do it
- Need to raise awareness on this threat

Malaria Policy Advisory Group statement on the **urgent need to address the high prevalence of *pfhrp2/3* deletions** in the Horn of Africa and beyond

World Health Organization

Dec 2021

June 2022

- Need to create a space for interaction to mobilize peer support among stakeholders
- Followed-up with experts engaged in the Forum and conceptualized the creation of a new CoP

MESA Forum: *Responding to the threat of malaria parasites evading HRP2-RDTs*

> 450 registrants
> 60 countries
> 90 questions

March 2023

- Analyzed the main interests and requests expressed by the CoP members

Launch of the MESA **Community of Practice on *pfhrp2/3* gene deletions**

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

June 2023

WHAT we do / HOW we do it

ACTIVITIES

1. Share best practices, reference materials (e.g. protocols, SOPs, training) and relevant **resources** from various stakeholders

IMPLEMENTATION

1. **CoP repository** in MESA **Resource Hub*** (*stay tuned for updates in the new MESA web to be launched soon)

WHAT we do / HOW we do it

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2. **Provide updates on new developments and advances**

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2. **CoP newsletters and social media**

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3. **Gather questions from NMPs and researchers, and channel these to the pool of experts who have volunteered to support sharing of knowledge within the CoP**

IMPLEMENTATION

1. CoP repository in MESA Resource Hub* (*stay tuned for updates in the new MESA web to be launched soon)

2. CoP newsletters and social media

3. **CoP e-mail address for communication to share questions, concerns and feedback** (hrp2.mesacop@isglobal.org)
   FAQs sheets regularly prepared and/or updated

WHAT we do / HOW we do it

ACTIVITIES

1. Share best practices, reference materials (e.g. protocols, SOPs, training) and relevant resources from various stakeholders

2. Provide updates on new developments and advances

3. Gather questions from NMPs and researchers, and channel these to the pool of experts who have volunteered to support sharing of knowledge within the CoP

4. **Organize events to provide updates and facilitate thematic discussions**

IMPLEMENTATION

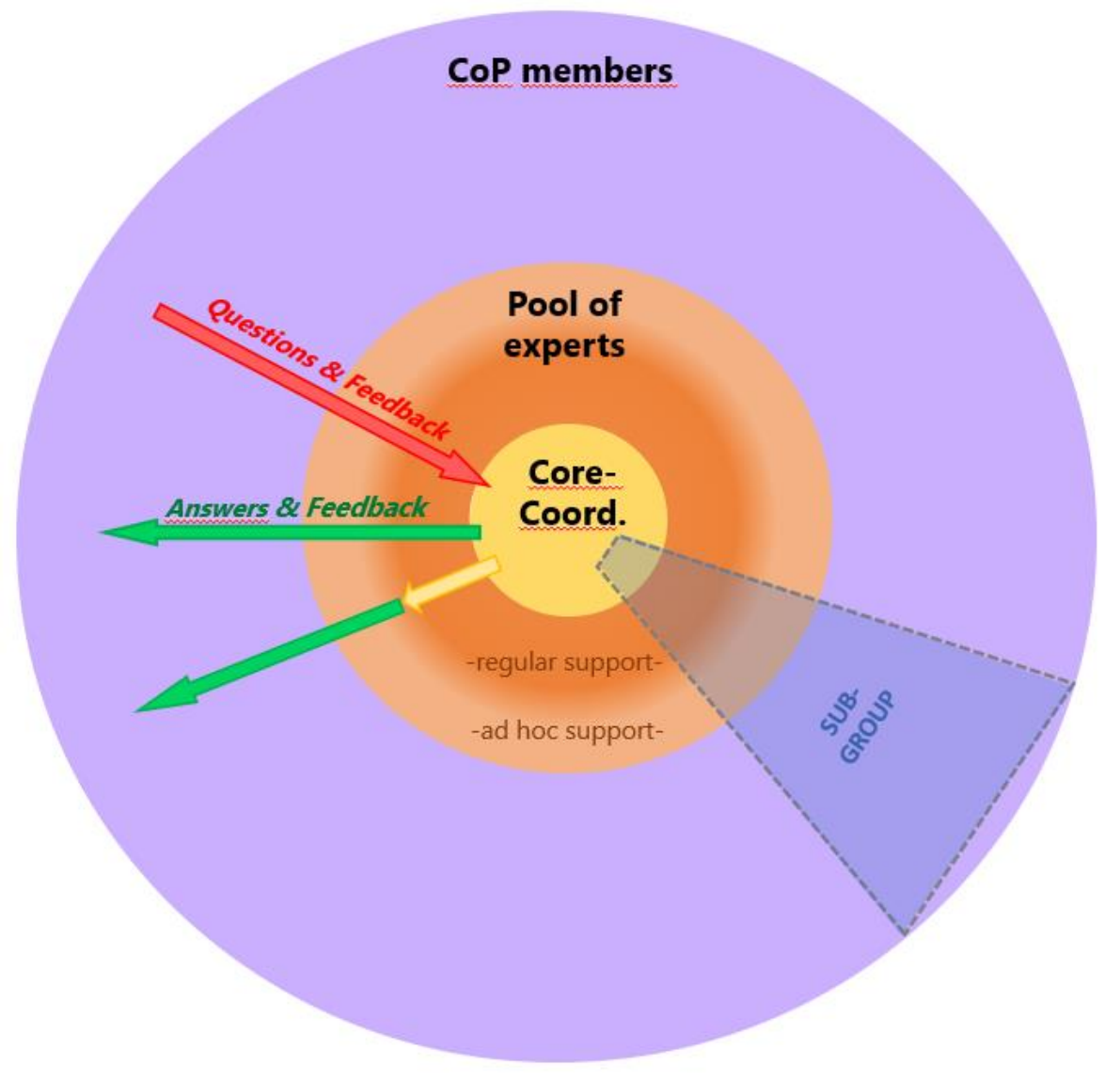
1. CoP repository in MESA Resource Hub* (*stay tuned for updates in the new MESA web to be launched soon)

2. CoP newsletters and social media

3. CoP e-mail address for communication to share questions, concerns and feedback (hrp2.mesacop@isglobal.org)
   FAQs sheets regularly prepared and/or updated

4. **Open Forums; CoP working group sessions (thematic, language specific); Trainings**

WHO: creation of the CoP



CoP CORE GROUP

Experts who contributed to the creation:

- Deus Ishengoma (NIMR, Tanzania) - *Chair*
- Dionicia Gamboa (UPCH, Peru)
- Eric Rogier (CDC, USA)
- Khalid Beshir (LSHTM, UK)
- Bosco Agaba (NMCP, Uganda)
- Jane Cunningham (WHO, Switzerland)
- Qin Cheng (ADFMIDI, Australia)
- Mateusz Plucinski (CDC/PMI, USA)
- MESA team - *Coordination*

WHO: CoP members profile

- **178 members** from **49 countries***

	N	%
Africa	118	*67%*
Asia	19	*10%*
North America	13	*8%*
Europe	16	*9%*
Latin America	9	*5%*
Oceania	3	*2%*

Most represented:

➔ Tanzania, Nigeria, Ethiopia, Cameroon

➔ India, Pakistan

➔ USA

➔ Spain

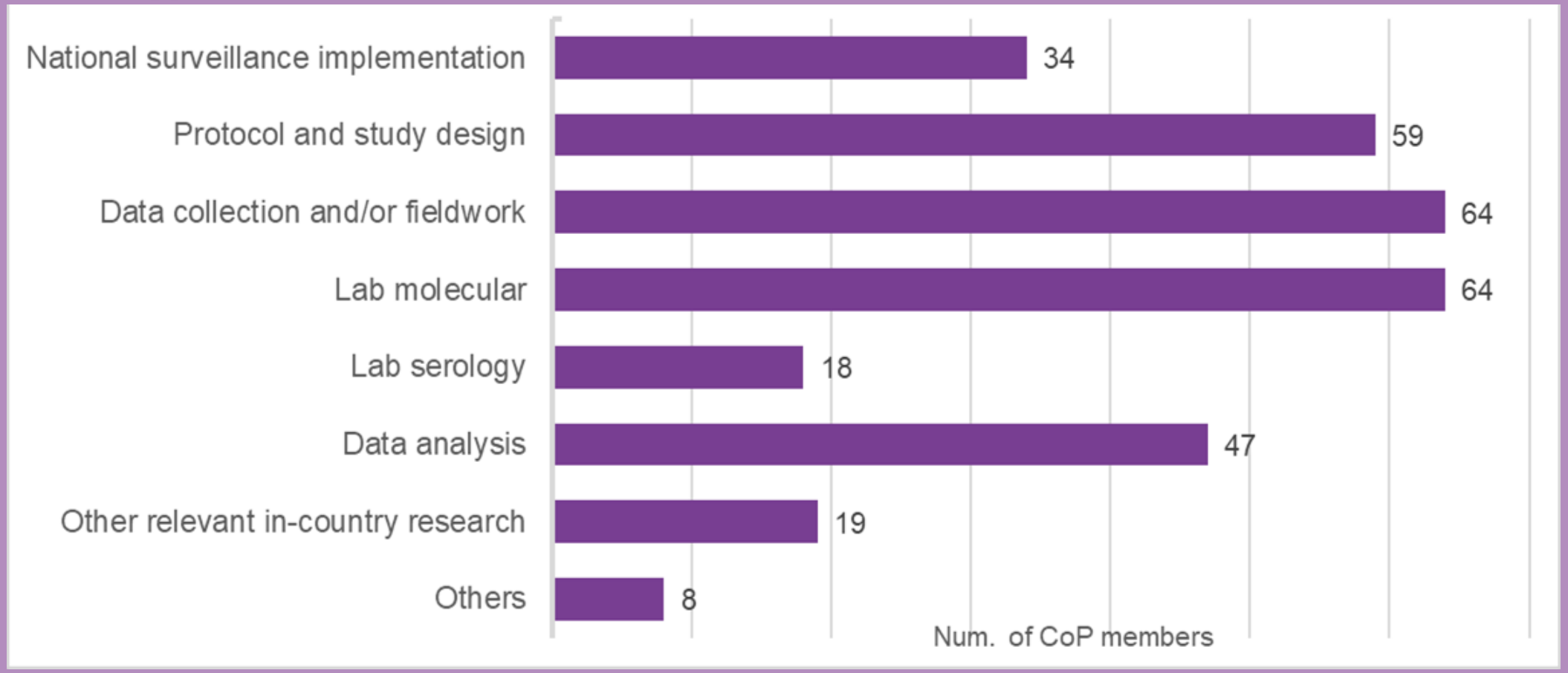
➔ Brazil, Peru

➔ Australia

**as of 30th May 2023*

WHO: CoP members profile

- 52% have been (or are currently) involved in *pfhrp2/3*-deletion surveillance
- Which type of activities are the CoP members involved in?



FOR WHAT: members' requests & interests

- Suggestions from the CoP members on what the CoP should address:

TOPICS of INTEREST

1. Implementation of surveys

2. Standardized laboratory procedures

3. Genomics/molecular epidemiology

4. Non-HRP2 based RDTs

5. Relation with other surveillance activities (e.g. drug resistance)

REQUESTS / EXPECTATIONS

★ Support in interpreting data, policies & guidelines

★ Lobby for funding

★ Organize (virtual or physical) events for more orientation

★ Capacity building

★ More involvement of National Programs

What's in stall for the CoP?

- Compilation of FAQs
- Repository of resources (videos, protocols, SOPs, documents as benchmarking resources to prevent duplication of efforts)
- Convening thematic sessions to address requests from the CoP members
- Networking
- In person gatherings
- Direct e-mailing: hrp2.mesacop@isglobal.org
- Training sessions

Acknowledgements

MESA is supported by a grant from the Bill & Melinda Gates Foundation

World Health Organization

Pool of experts who have volunteered to support the activities of the CoP

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

CoP Core Group members:

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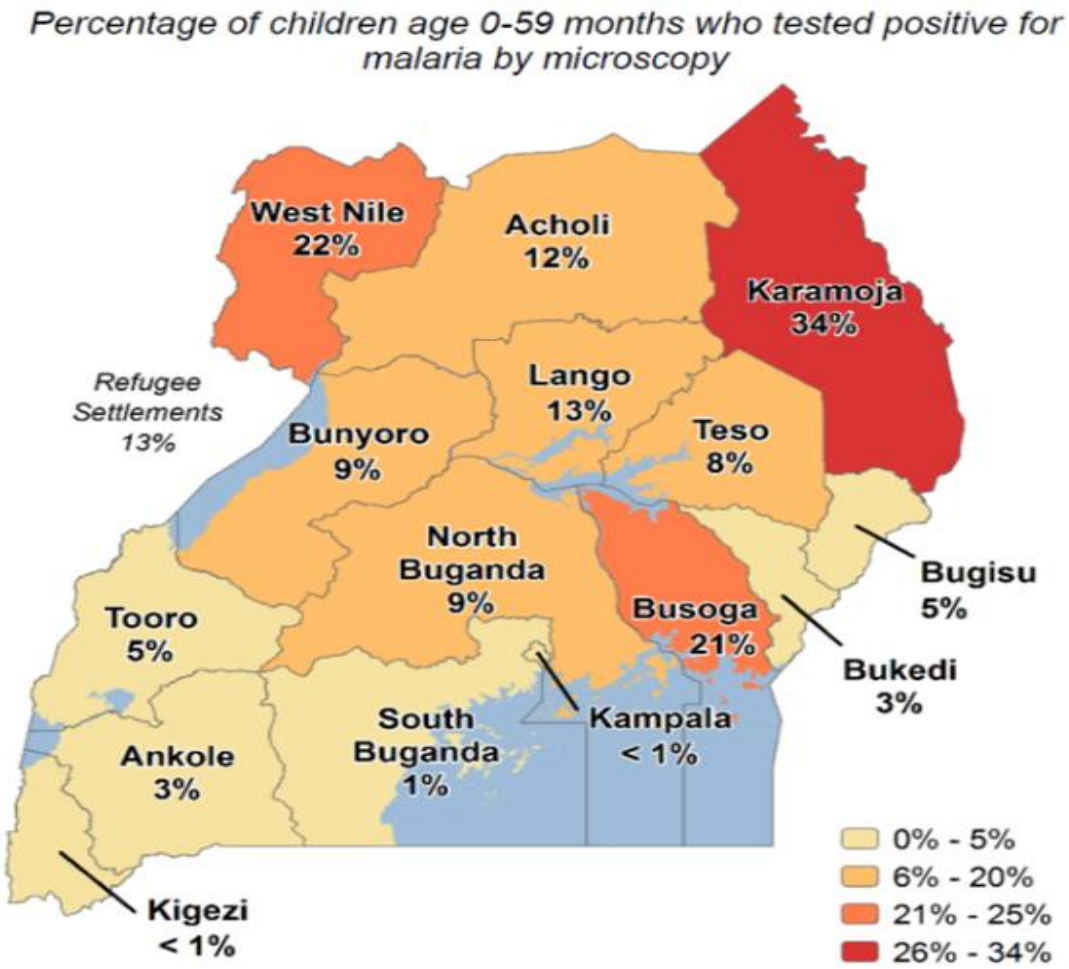
IDRC
INFECTIOUS DISEASES RESEARCH COLLABORATION
Excellence in Infectious Diseases Research

pfhrp2 and pfhrp3 surveys; Field Implementation experience from Uganda

Dr. Agaba Bosco (PhD)
NMCD, Uganda

BACKGROUND

- Malaria remains public health problem
- Accurate diagnosis- is a key intervention
- RDTs are the main diagnostic tools for malaria
- RDTs are threatened by *phrp*2 gene deletions
- WHO recommends surveillance of deletions
- No routine surveillance system for deletions

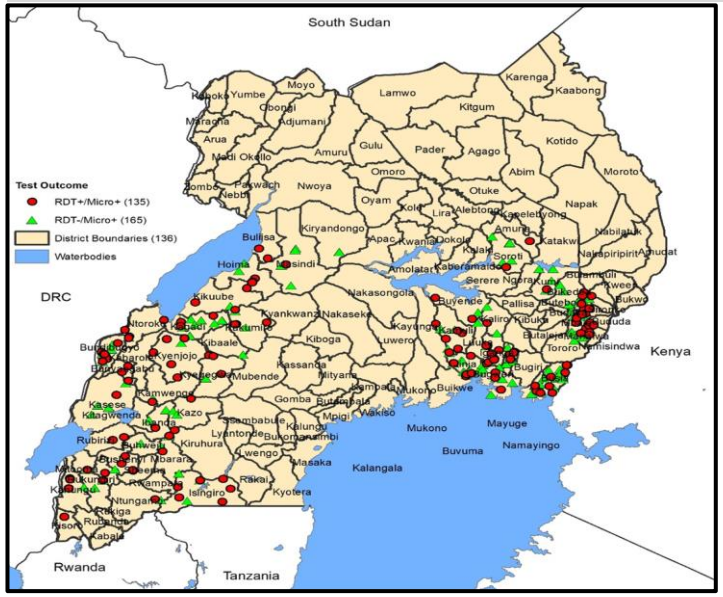


Main pfhrp2/3 Survey Objectives

- Estimate prevalence of suspected false-negative HRP2 RDT results among symptomatic patients with *P. falciparum*
- Determine prevalence of *pfhrp2/3* gene deletions among symptomatic falciparum patients with a false negative RDT
- Estimate prevalence of non-*falciparum* species that can lead to false negative results with HRP2-RDTs

What is known?- Brief snapshot of the surveys

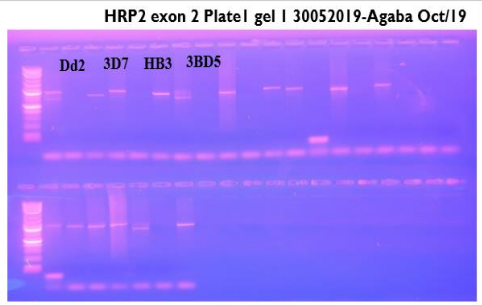
First Hrp2 deletions survey (n=300)

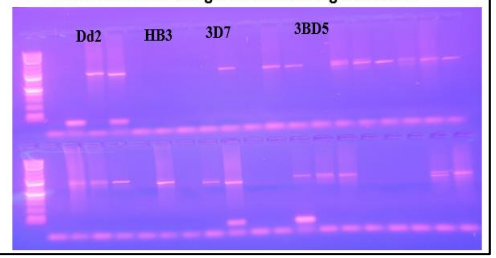


Used Conventional PCR in 1st Survey

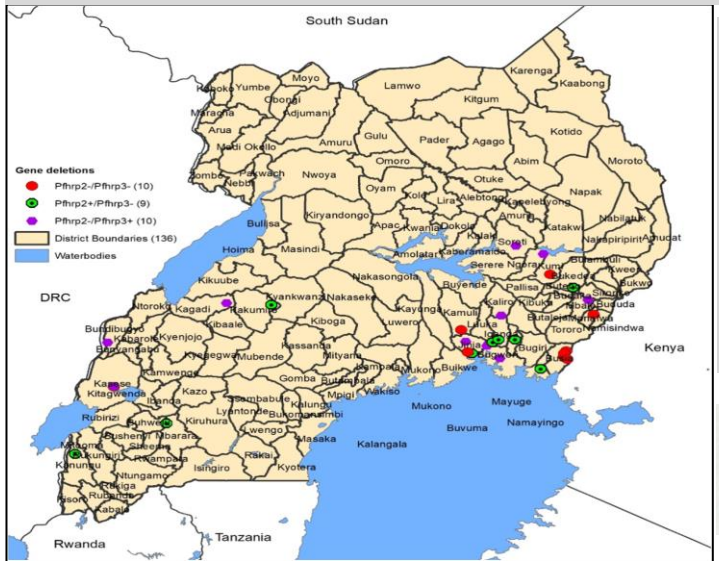
***pfhrp2/3* assay controls**

Parasite strain	*Pfhrp2* status	*Pfhrp3* status
P. f 3D7	(+)	(+)
P. f Dd2	(-)	(+)
P. f HB3	(+)	(-)
P. f 3BD5	(-)	(-)
Human negative	(-)	(-)





Distribution of deletions in survey 1



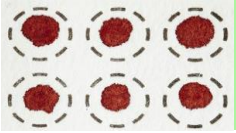
Summary of Results from 1st Survey

Overall: 29 (9.7%; CI: 6.6-13.6)

pfhrp2-/pfhrp3+ : 10 (3.3%; CI: 1.6- 6.0)

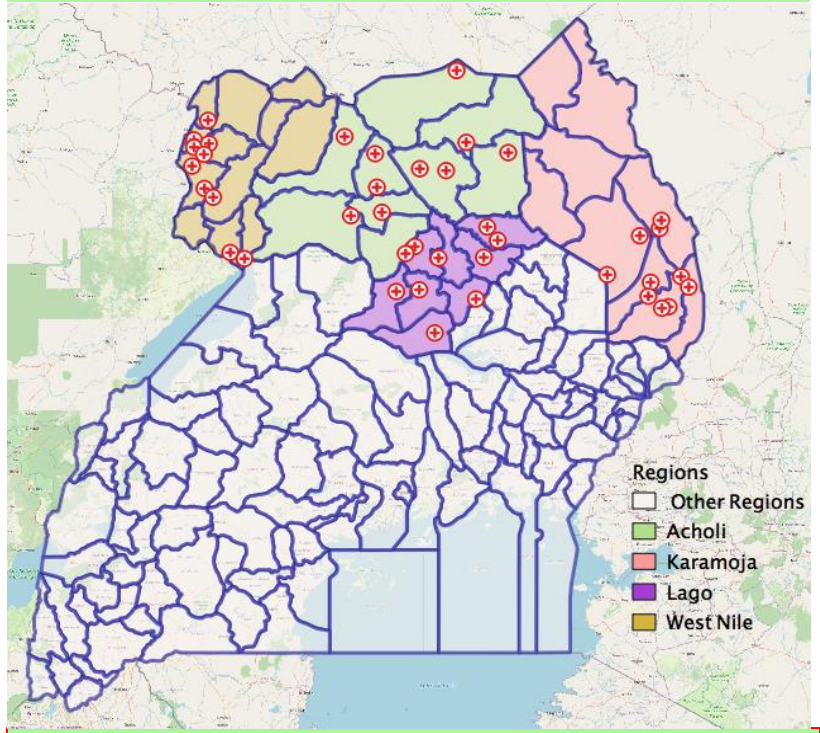
pfhrp2+/pfhrp3- : 9 (3.0%; CI: 1.4- .5.6)

pfhrp2-/pfhrp3- : 10 (3.3%; CI: 1.6- 6.0)



Most false (-) not due to deletions but; Low density, non-Pf species

2nd Survey- sites across 4 Regions



- pfhrp2 and pfhrp3 status determined using multiplex qPCR (Grignard et al 2020)
- amplifies a fragment each of *pfhrp2, pfhrp3, pfldh* and human tubumin (*htb*) genes simultaneously
- Report writing on-going

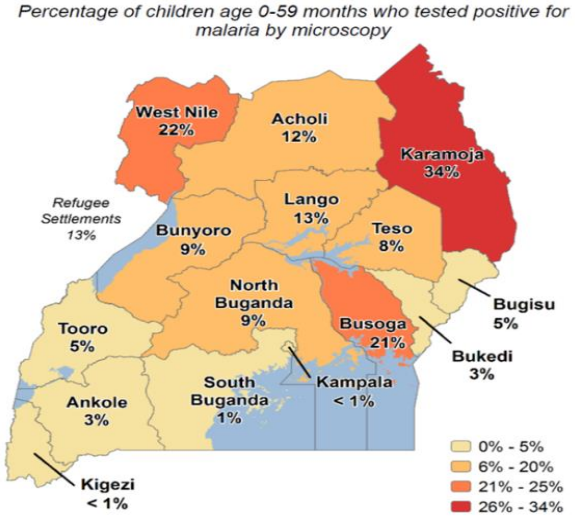
Sequence of steps followed to implement

- Stakeholder engagement
- Identify survey areas/coverage (if not national survey)
- Quantify the need (resources, supplies, etc)
- Resources mobilization
- Site selection
- Protocol (IRB, investigators, etc)
- Data tools (questionnaires, consent, translations)
- Constitute survey teams
- Site initiations
- Collection-data, samples
- Lab testing – reference Lab if available or shipment
- Data analysis & Report
- Dissemination to NMCP, partnership, publication

Practical Considerations that enabled success

1

Positioning of sites- concerns of false negative, ensuring representativeness



2

Interchange of RDTs if using two RDTs

1.	Barcode/Patient ID	*Place label*
2	Health centre	*Pre-entered for each health centre on printed form or combined with survey ID*
3.	Name of health worker/ lab assistant	
4.	Date of visit	Day____Month____Year____
5.	*Pre-entered for each health centre on printed form:* RDT 1 (must include HRP2- National programme RDT) a. Name: b. Product code: c. Lot number: d. Expiry date: e. Target antigens: 1. T1: 2. T2: 3. T3:	 Box 1: Control / P.f HRP2: + / - ; + / - *Circle correct result in each box above.* Circle result of RDT: 1. Negative 2. *P. falciparum*
6.	RDT 2 (survey RDT) a. Name: b. Product code: c. Lot number: d. Expiry date: e. Target antigens: 1. T1: 2. T2: 3. T3:	 Box 2: Control / T2 Pf-LDH: + / - / T1 HRP2: + / -

3

DBS- Use correct filter paper, Train field techs on spots

Microscopy competency if using Blood smears

4

5

Work within existing structures

Survey supervision

6

Potential Challenges

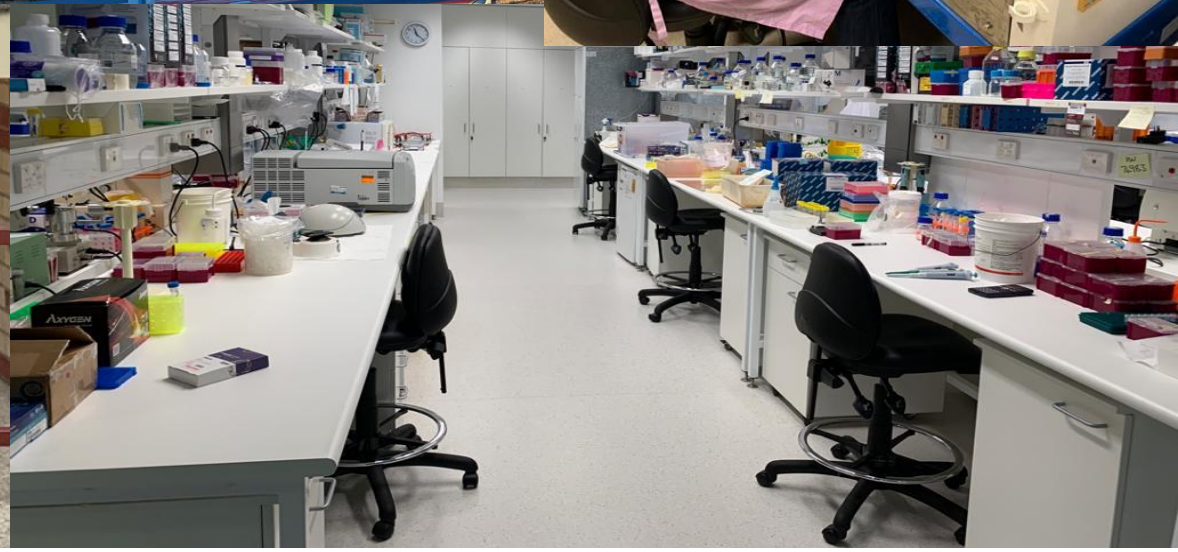
- Integration into routine surveillance
- Capacity
- Surveys largely remain in "project" mode
- Lengthy processes for MTA for those intending to ship
- communicating hrp2 deletion results where prev <5%
- Introduction of alternative tests alongside HRP2 in areas with deletions (guidelines, training, Supply chains, etc)

Deployment of alternative tests alongside HRP2 in requires efficient distribution system

Lessons

- Top-up for health workers motivated government staff
- Use of existing capacity within the region
- Pooled procurement of supplies
- Resources- both grants and domestic resource
- Adhere to WHO protocol
- Inclusion of NMCP investigators on survey protocol

Collaborating Institution



Many thanks to

Uganda Team

Prof. Moses Kamya

Dr. Emmanuel Arinaitwe

Dr. Isaac Sewanyana

IDRC

NMCD

Collaborating Team

- Prof. Qin Cheng
- Dr. Jane Cunningham

Funder

- BMGF
- GOU
- FIC

FOR GOD AND MY COUNTRY

World Health Organization

Australian Army Malaria Institute

IDRC INFECTIOUS DISEASES RESEARCH COLLABORATION

BILL & MELINDA GATES foundation

WE BUILD FOR THE FUTURE

Pfhrp2/3 Genotyping: Techniques and Approaches

Dr Khalid B Beshir
Assistant Professor
Faculty of Infectious and Tropical Diseases

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

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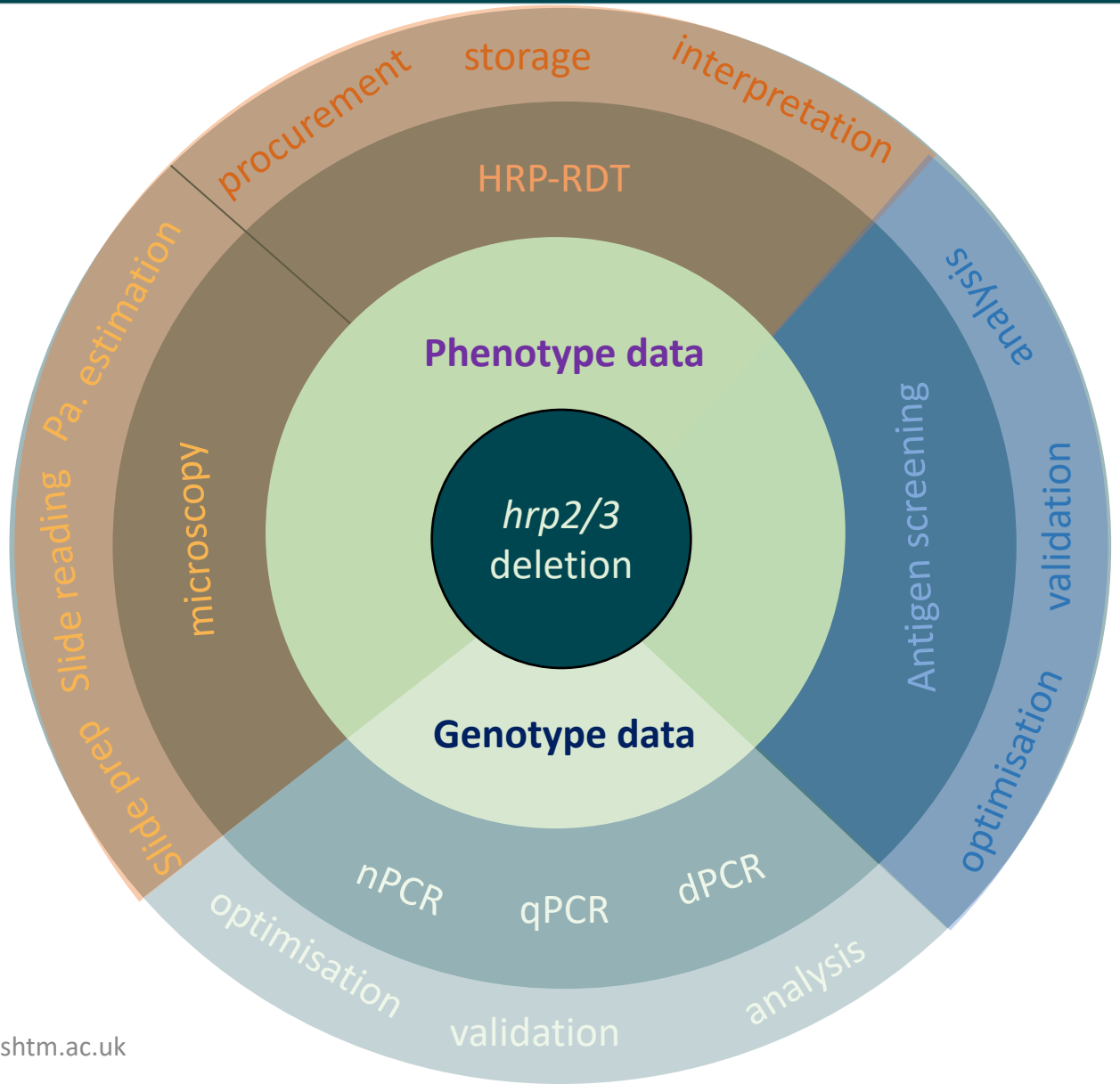
Pfhrp2/3 Genotyping: Techniques and Approaches

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Techniques



Approaches

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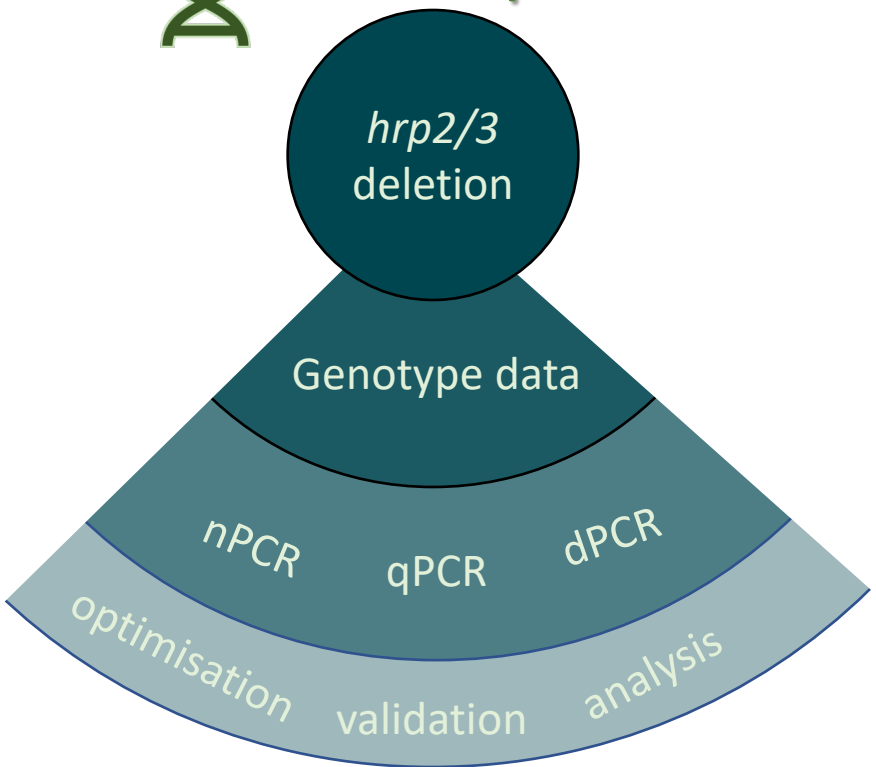
Pfhrp2/3 Genotyping: Techniques and Approaches

Confirm quality of DNA targeting single copy gene

hrp2/3 detection and characterisation

NGS - targeted or whole genome

Techniques



PCR confirmation of *P. falciparum* and speciation

Microsatellite typing

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Pfhrp2/3 Genotyping: Techniques and Approaches

How can we find ***hrp2/3*** **deletions?**

Pfhrp2/3 Genotyping: Techniques and Approaches

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

How can we find ***hrp2/3* deletions?**

RDT negative

can also be caused by

factors other than

***hrp2/3* deletion**

hrp2/3
deletion

Pfhrp2/3 Genotyping: Techniques and Approaches

How can we find ***hrp2/3* deletions?**

RDT negative

can also be caused by

factors other than

***hrp2/3* deletion**

hrp2/3 deletion

- RDT device related issues such as storage, transport etc
- Parasite factors such as low parasite density, very high parasite density

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Pfhrp2/3 Genotyping: Techniques and Approaches

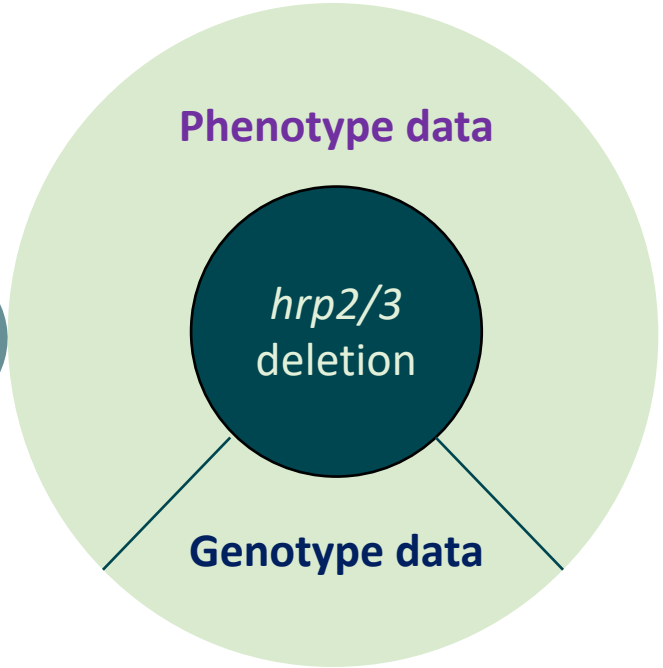
How can we find ***hrp2/3* deletions** that cause **HRP2-RDT negative?**

RDT negative

can also be caused by

factors other than

***hrp2/3* deletion**



Percentage of

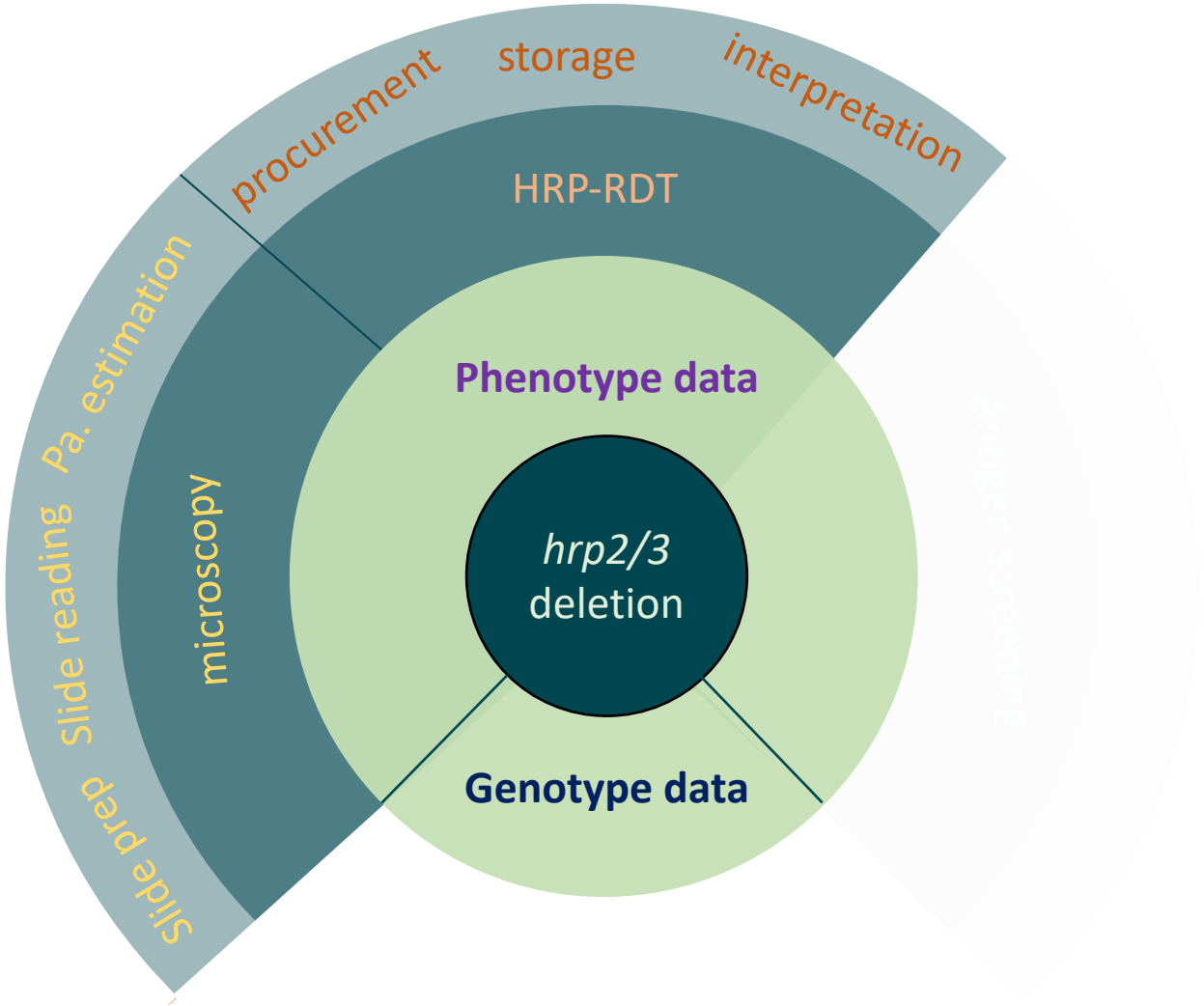
HRP2-RDT negative

caused by

***hrp2/3* deletion**

Pfhrp2/3 Genotyping: Techniques and Approaches

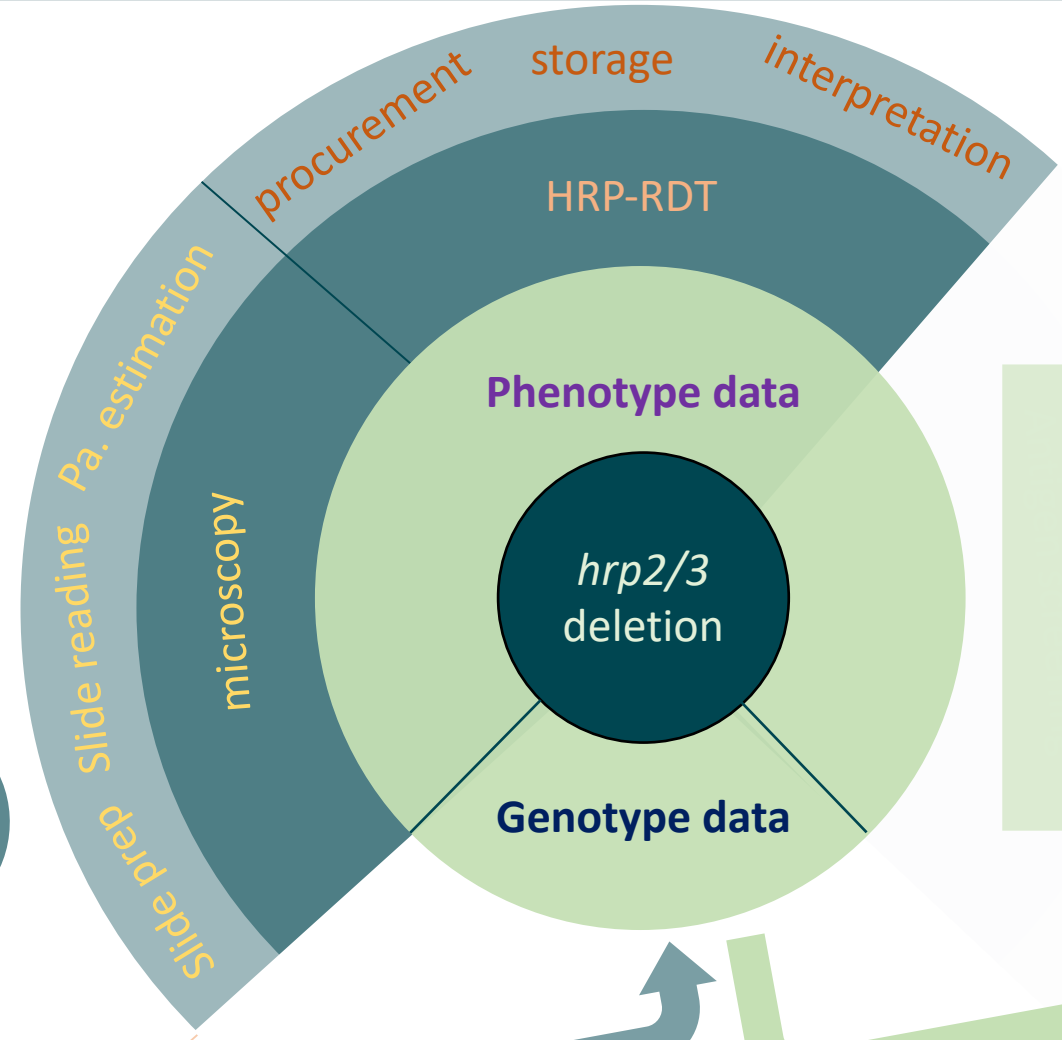
Field hrp2/3 deletion surveillance



Pfhrp2/3 Genotyping: Techniques and Approaches

Field hrp2/3 deletion surveillance

Mic and RDT discordant samples



Percentage of

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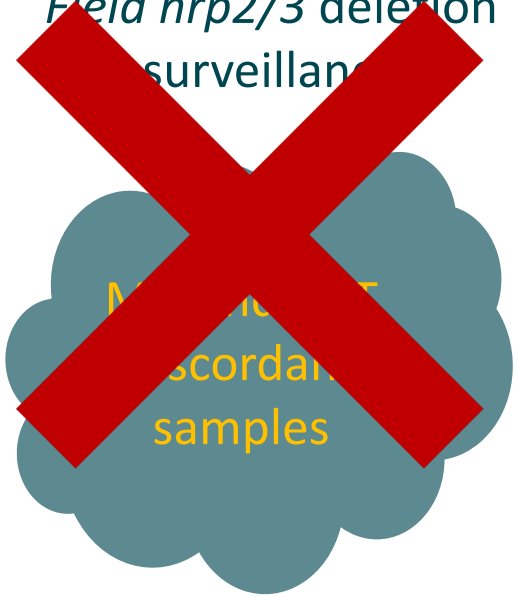
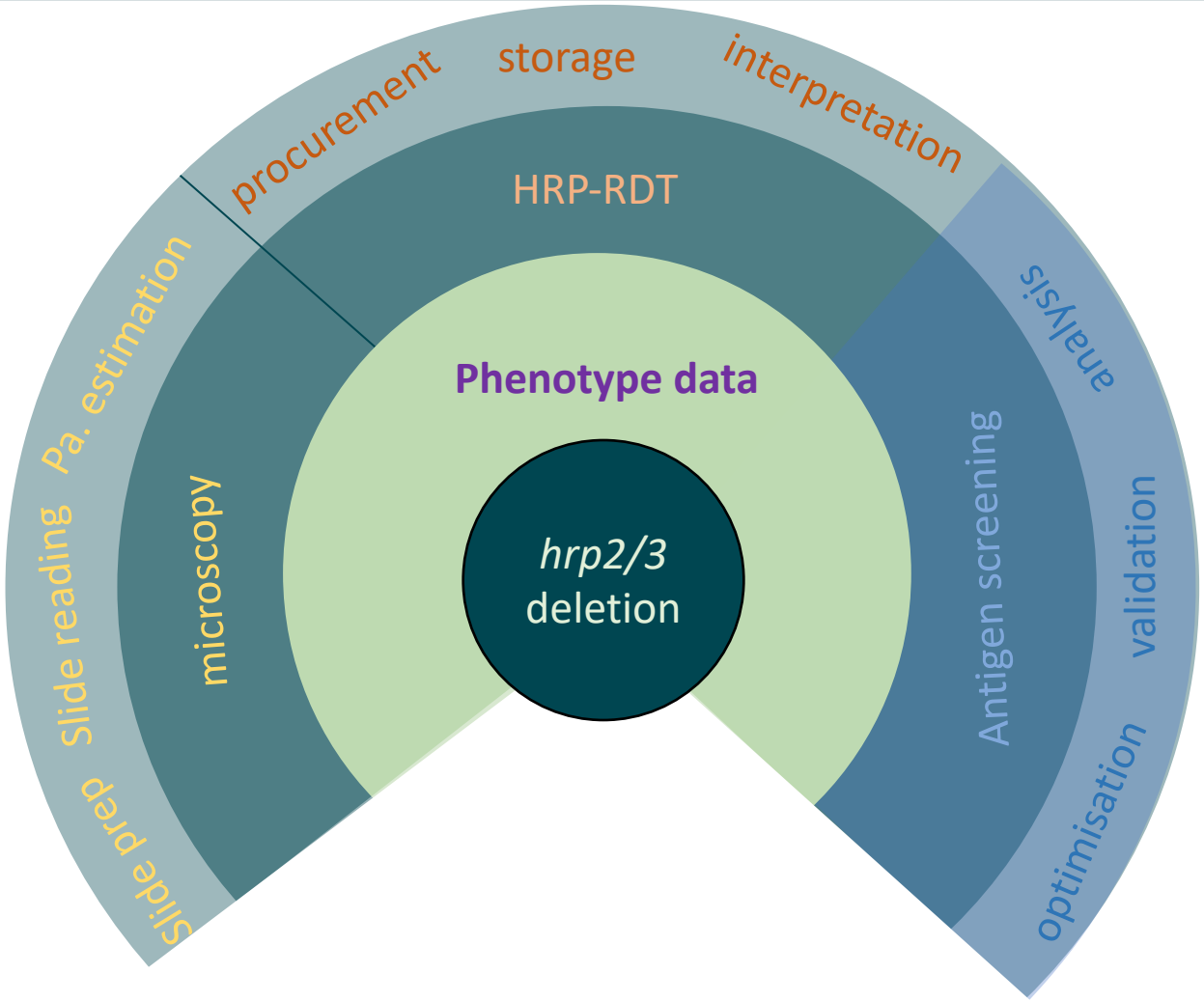
Pfhrp2/3 Genotyping: Techniques and Approaches

Field hrp2/3 deletion
surveillan





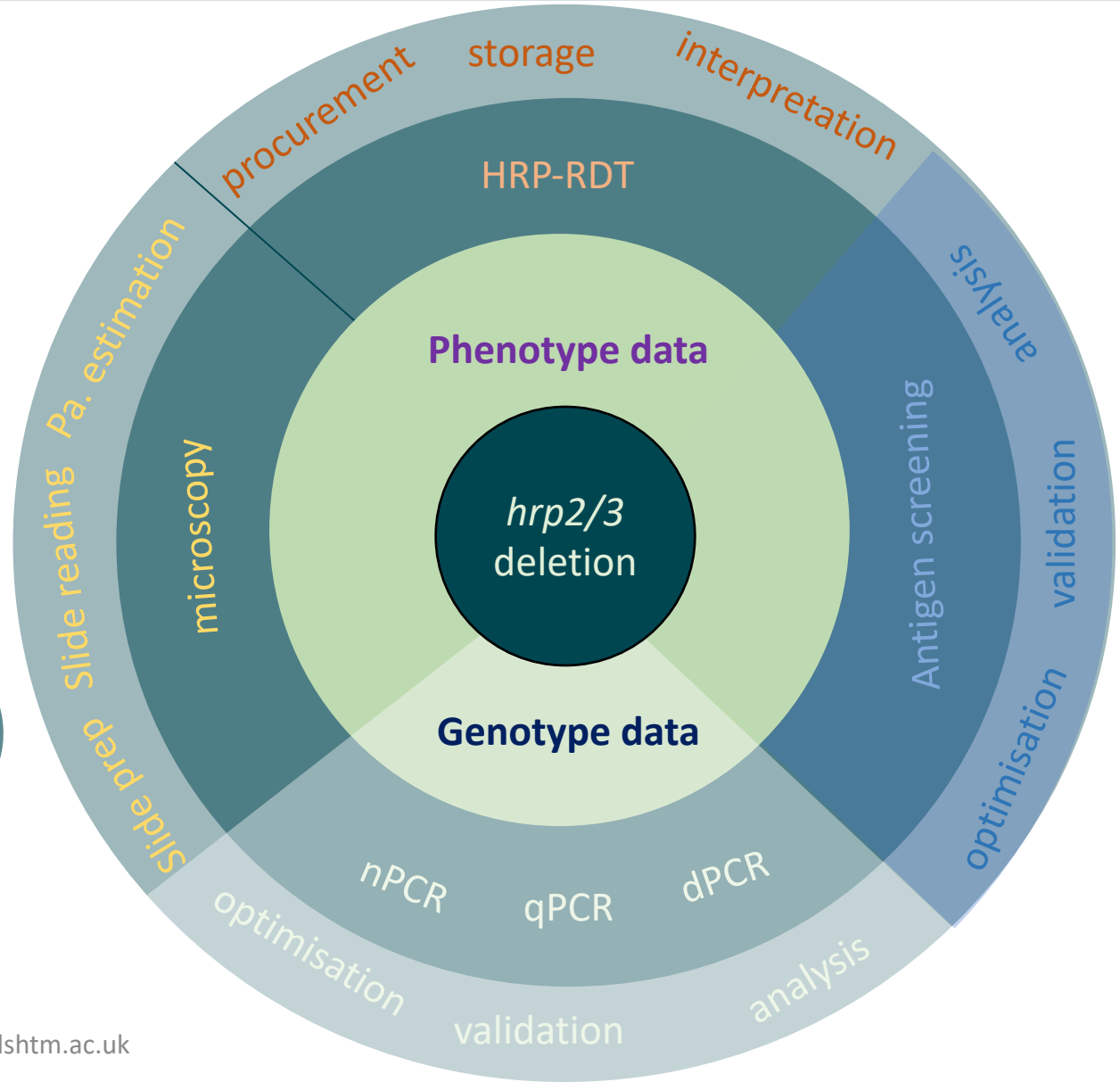
Use case (TES, PMC, SMC)
Or mic/RDT is not feasible

Post hoc laboratory assays

Pfhrp2/3 Genotyping: Techniques and Approaches

Field hrp2/3 deletion surveillance

Mic and RDT discordant samples



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Post hoc laboratory assays

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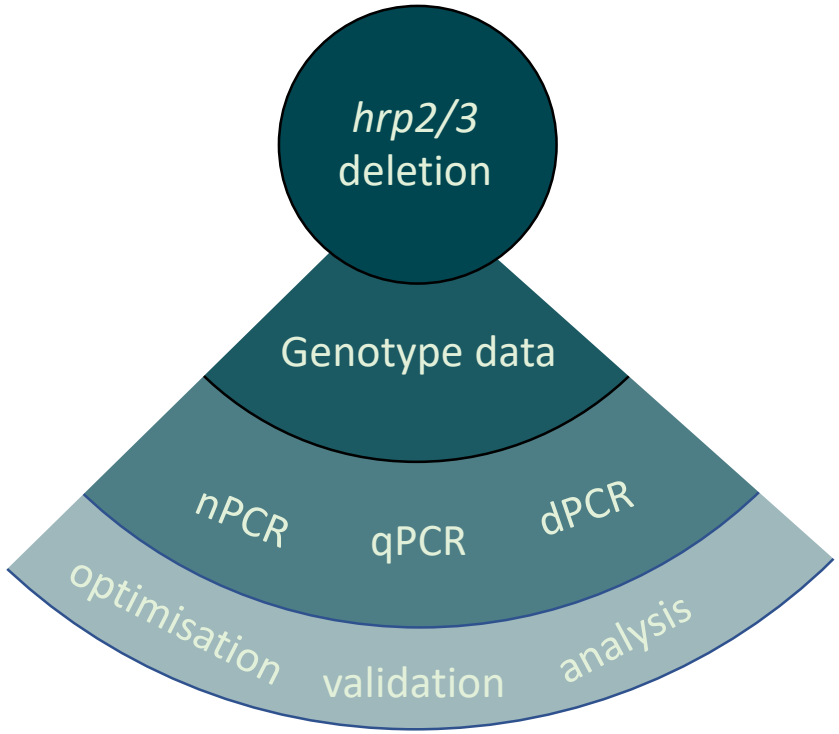
Pfhrp2/3 Genotyping: Techniques and Approaches

Methods available

1. Nested PCR (nPCR)
2. Quantitative PCR (qPCR)

} Validated and widely used by WHO network

3. Digital PCR (dPCR)
   - A promising technology, particularly for multi-*hrp2/3* clonal samples
   - Requires validation
   - Comparison in speed, simplicity and interpretation
4. Amplicon sequencing – emerging method
   - Similar to nested PCR but based on sequencing reads
   - Requires validation



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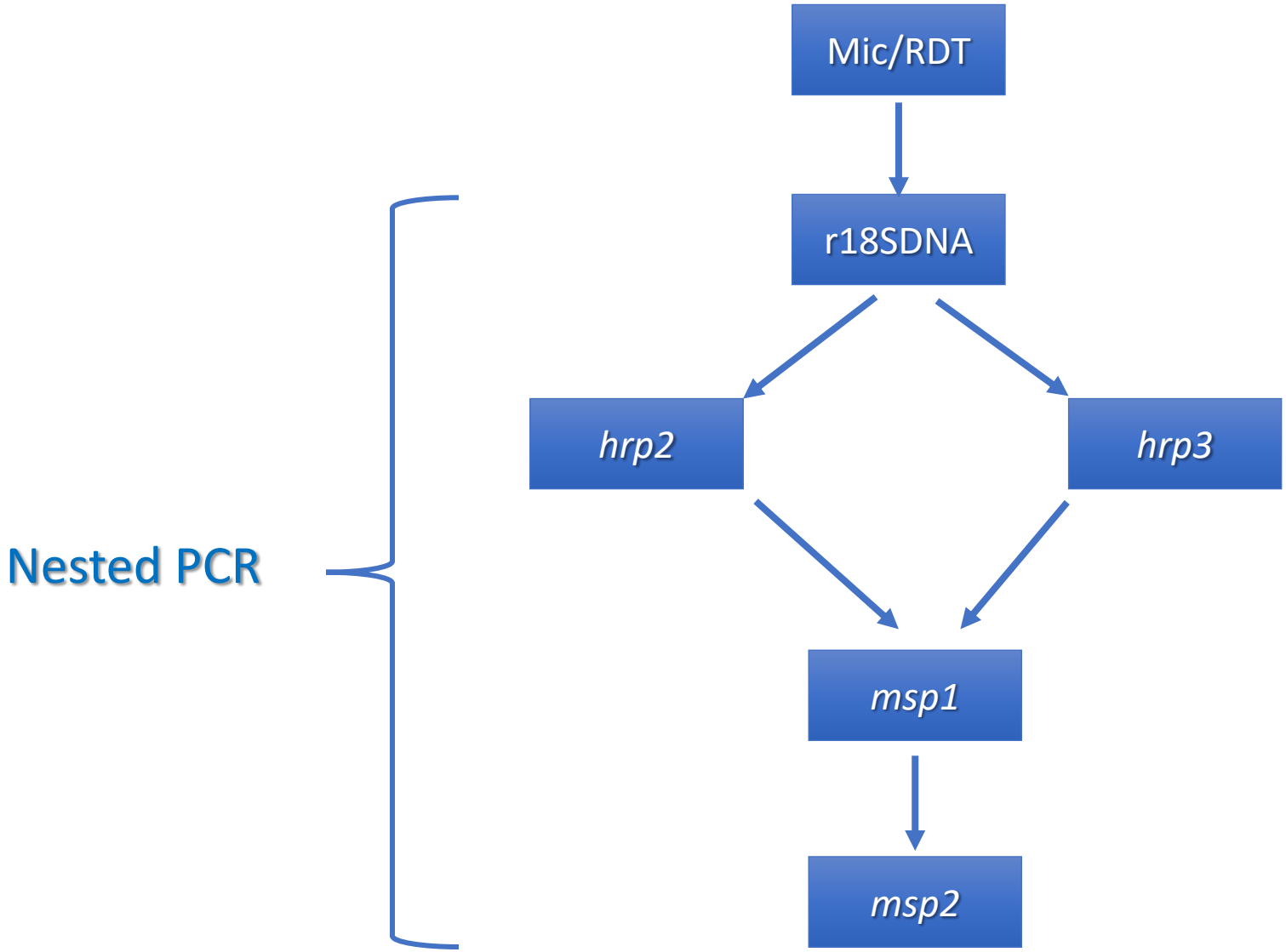

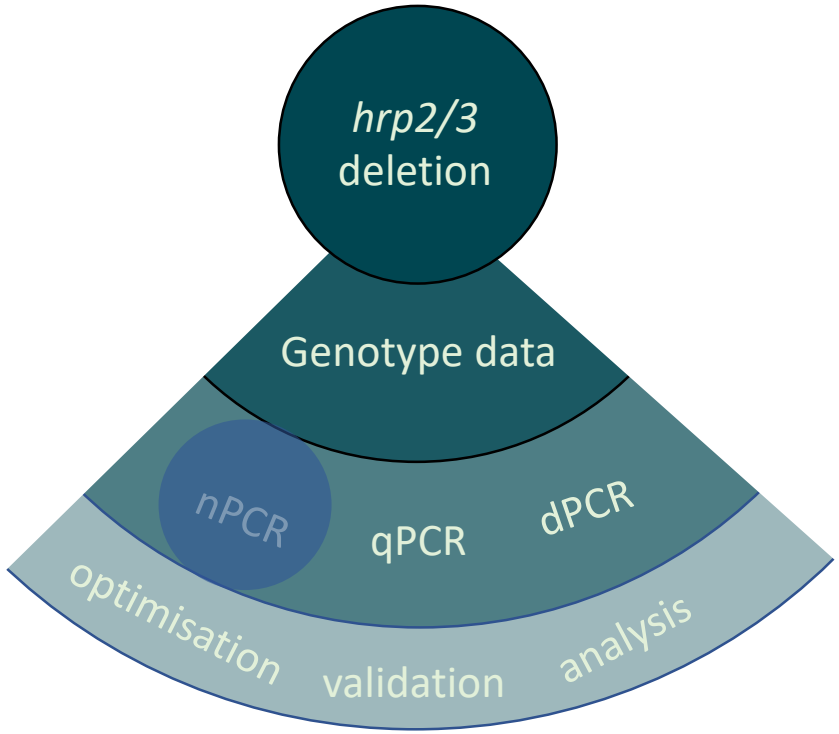

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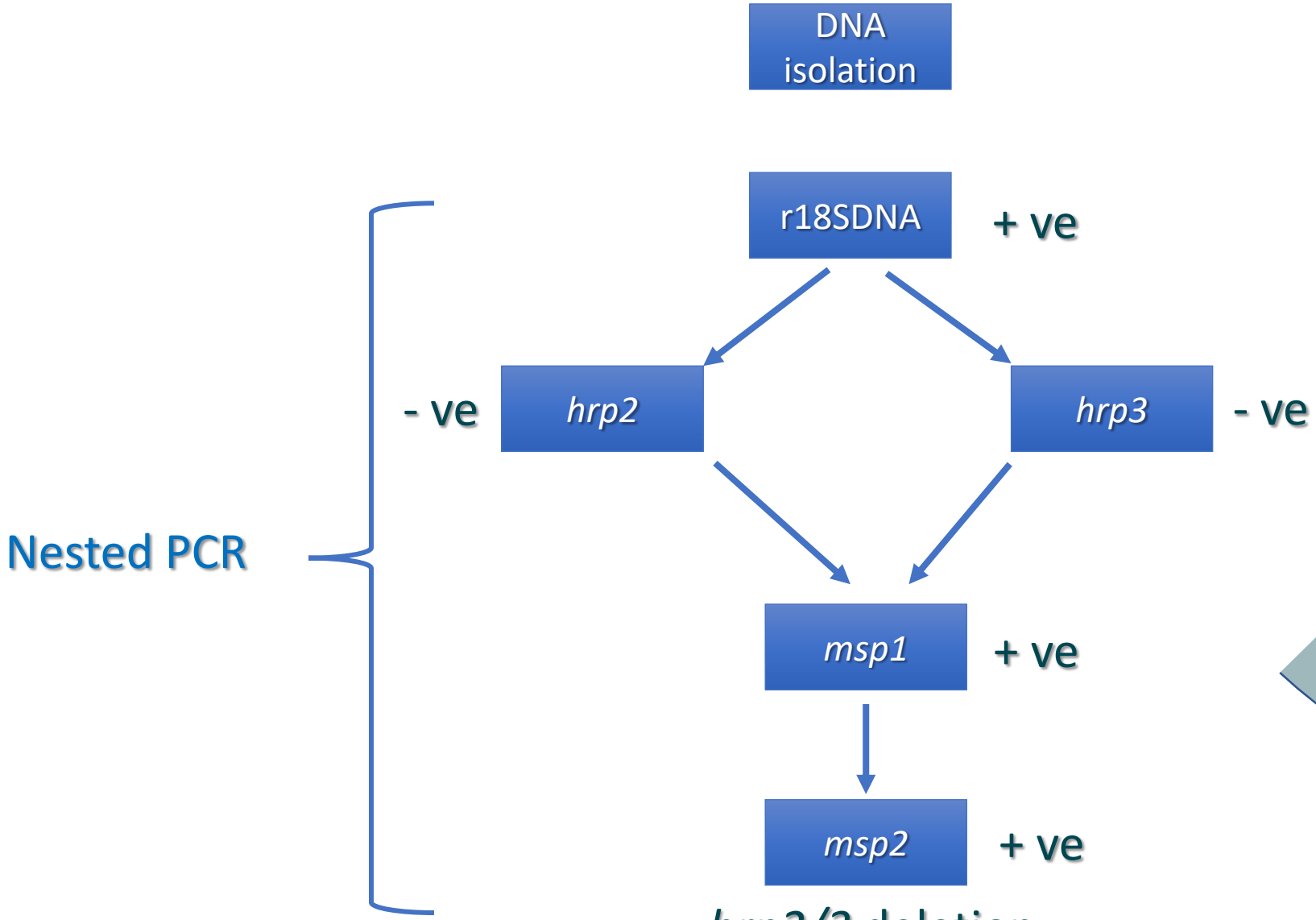
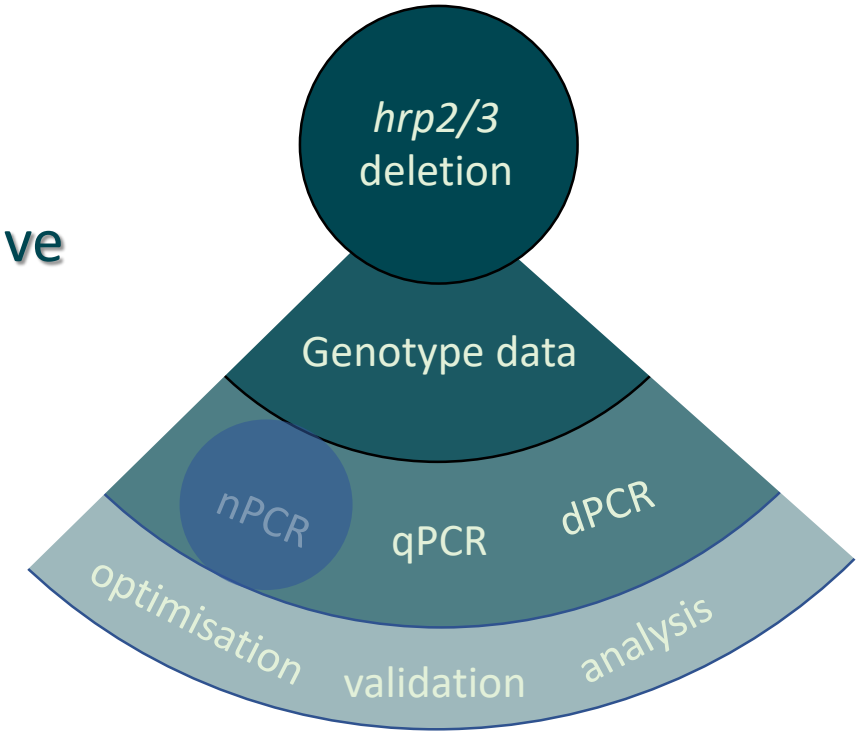
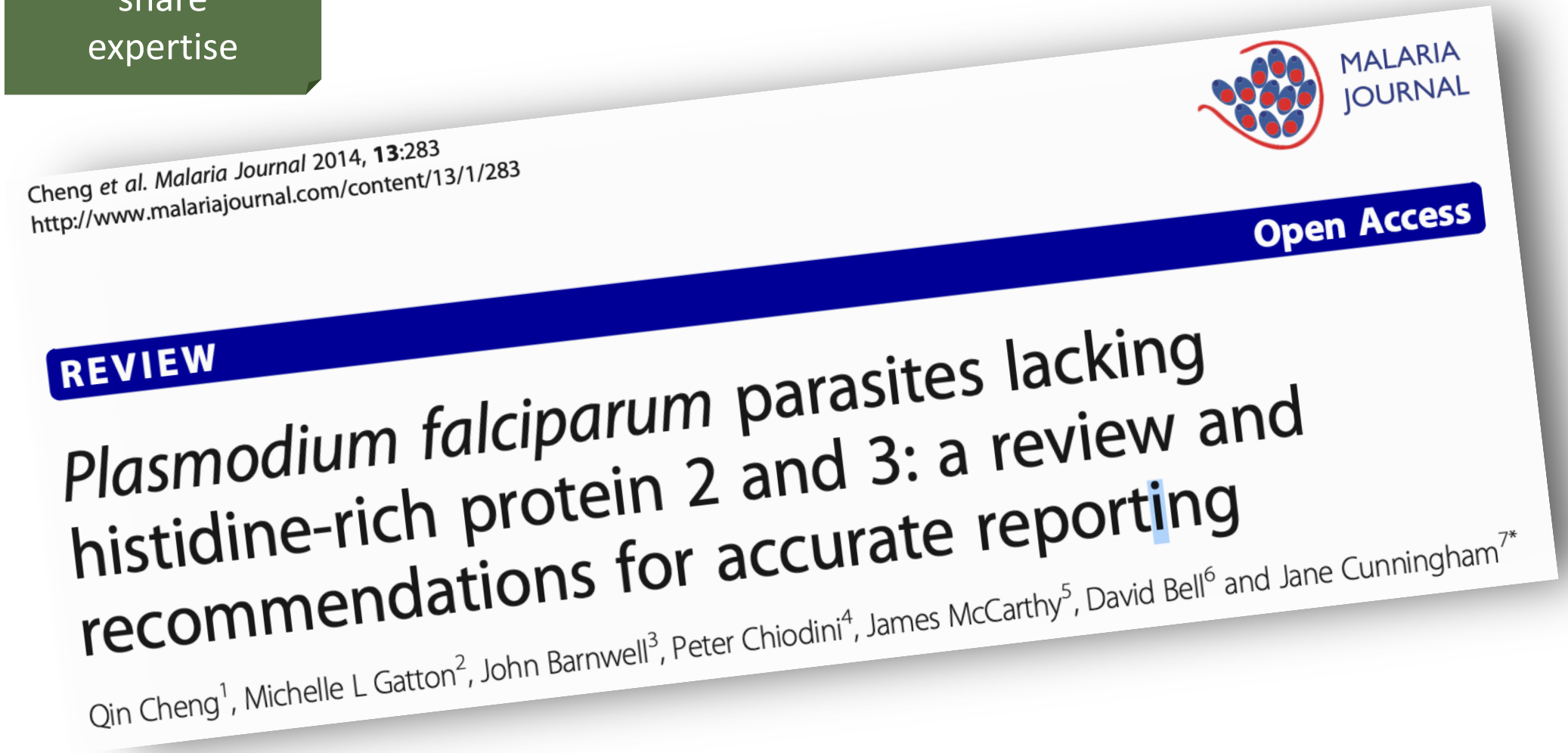
Pfhrp2/3 Genotyping: Techniques and Approaches





Pfhrp2/3 Genotyping: Techniques and Approaches

share expertise



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

MALARIA JOURNAL

Open Access

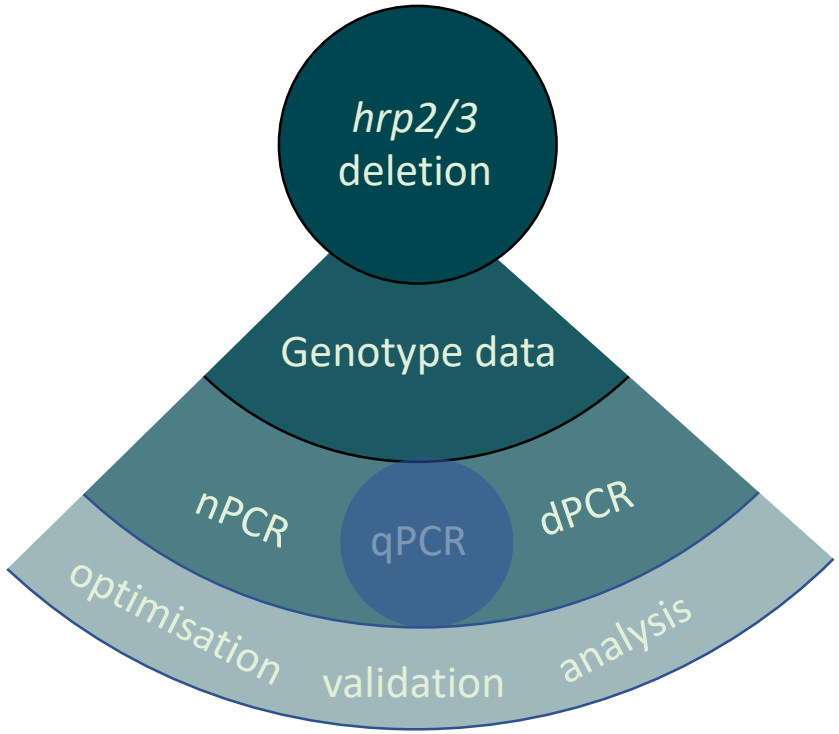
REVIEW

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

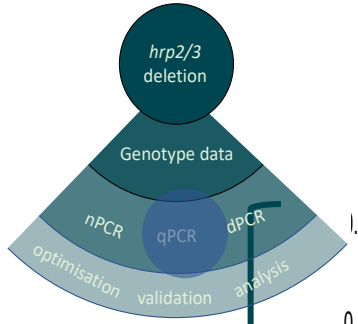
Qin Cheng[1], Michelle L Gatton[2], John Barnwell[3], Peter Chiodini[4], James McCarthy[5], David Bell[6] and Jane Cunningham[7*]

Quantitative PCR (qPCR)

1. **Four sets of primers targeting**
   - **One human gene (*HumβTub)***
   - **Three parasite genes (*ldh, hrp2 and hrp3)*)**
2. **Four probes targeting each gene**
3. **All reagents added in one tube per sample**
4. **No second round of PCR**

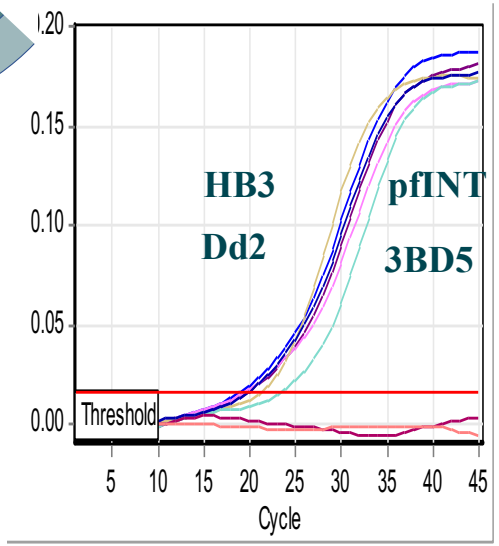


Pfhrp2/3 Genotyping: Techniques and Approaches

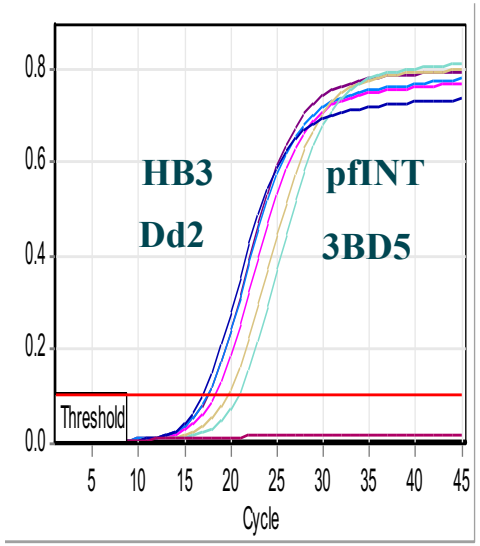


qPCR

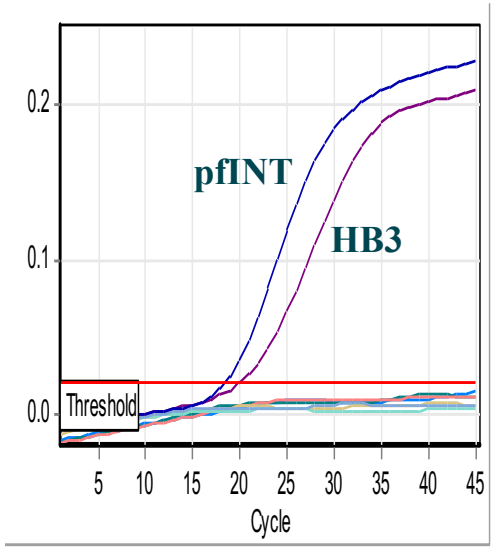
Channel CY5
Human DNA



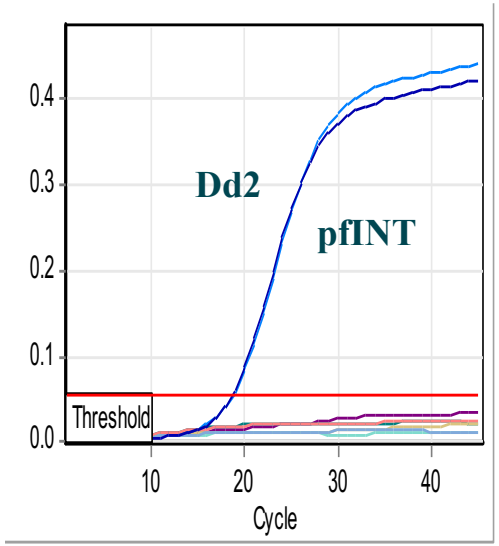
Channel ROX
***ldh* DNA**



Channel FAM
***hrp2* DNA**

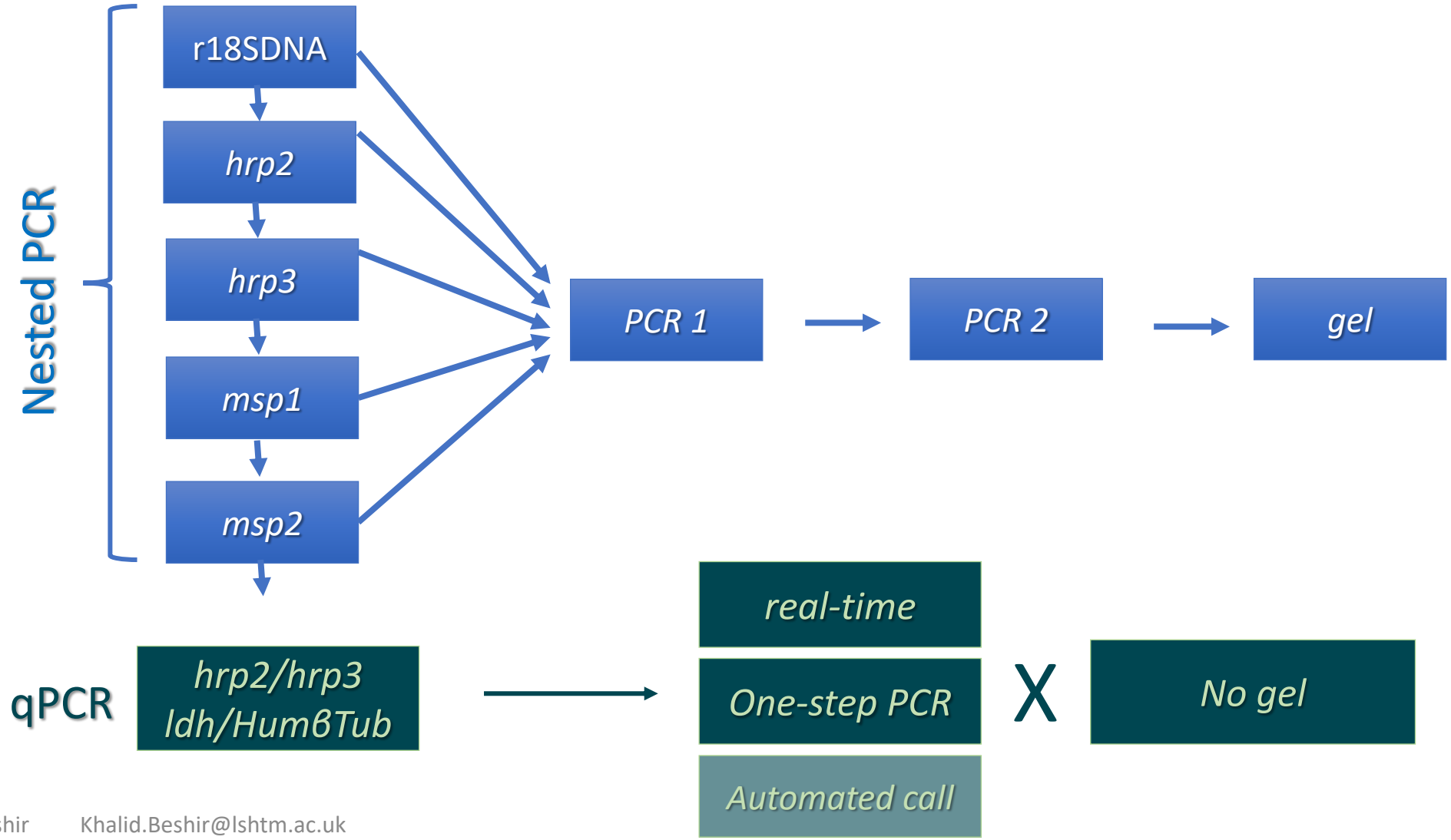


Channel JOE
Human DNA

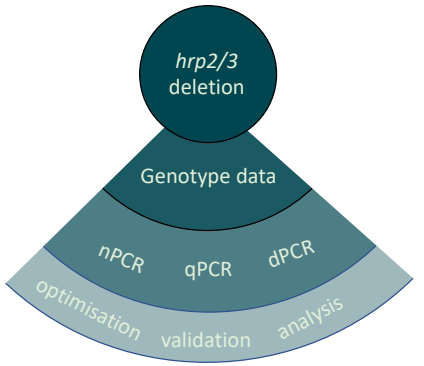


No deletion	**Positive**	**Positive**	**Positive**	**Positive**
Deletion	**Positive**	**Positive**	**Negative**	**Negative**
No parasite	**Positive**	**Negative**	**Negative**	**Negative**
No *hum* DNA (Invalid)	**Negative**	-	-	-
controls	**all**	**all**	**PfINT and HB3**	**PfINT and Dd2**

Pfhrp2/3 Genotyping: Techniques and Approaches



Pfhrp2/3 Genotyping: Techniques and Approaches



Nested PCR

- r18SDNA
- *hrp2*
- *hrp3*
- *msp1*
- *msp2*

Pipetting error → *PCR 1* (2 hrs x 5)

PCR inhibition → *PCR 2* (2 hrs x 5)

prone to contamination → *gel* (2 hrs x 5)

= 30 hours

qPCR — *hrp2/hrp3 ldh/HumβTub*

Human DNA detects error and PCR inhibition

= 2 hours

Pfhrp2/3 Genotyping: Techniques and Approaches

share expertise

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

Malaria Journal

Open Access

REVIEW

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*]

Pfhrp2/3 Genotyping: Techniques and Approaches

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

hrp2/3 molecular genotyping – practical considerations

- Optimisation
- Validation
- Positive controls
- Internal controls
- Negative controls
- WHO NAAT EQA

1. **Assay repeatability and reproducibility**
2. **Validation of new assays**
3. **Use of well-characterised *P. falciparum* samples for positive controls**
4. **Appropriate use of internal control (human DNA)**
5. **Inclusion of assay wells containing no template control (NEG)**
6. **Participation in WHO Malaria NAAT**

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WHO international lab network to support *pfhrp2/3* surveillance

- A geographically diverse labs with experience characterizing *pfhrp2/3* deletions and lab resources, participating on the WHO NAAT EQA
- Established in 2017, currently 7 labs, 2 under consideration
- Terms of reference
- Engage in tripartite agreements between WHO-Lab-survey country (MOH, research institute)
- WHO has some funding to support molecular and serological analysis and some of the labs also have funding sources
- Contact WHO to be directed to a lab, preferably at planning stage
  - Dr Andrea Bosman, bosmana@who.int;
  - Interim: Dr Qin Cheng, qin.cheng@defence.gov.au

Lab capability

Institute	Country	Lead	PCR/qPCR for Speciation	Molecular analysis to confirm gene deletions				Serological analysis to confirm lack of HRP2 expression		Other molecular tests	
				Conventional PCR	Multiplex qPCR	Digital PCR	WGS/ Genomics	ELISA	Bead-based assay	MOI/ Origin	K13 mutations
LSHTM	UK	Dr. Khalid Beshir	Y	Y	Y	N	Y	N	N	Y (not routinely)	Y
UNC	USA	Dr. Jonathan Parr	Y	Y	Y	N	Y	N	Y (not routinely)	Y	Y
ADFMIDI	Australia	Dr Qin Cheng	Y	Y (moved away)	Y	Y (not routinely)	N	Y	N	Y	Y
CDC	USA	Dr Eric Rogier/?	Y	Y	N	N	N	N	Y	Y	Y
UCAD	Senegal	Prof Daouda Ndiaye	Y	Y	Y	N	Y	Y	Y	Y	Y
UPCH	Peru	Dr Dionicia Gamboa	Y	Y	N	N	Y	Y	Y	Y	Y
NIMR	India	Dr Praveen Bharti	Y	Y	Y	N	Y	Y	N	Y	Y
AHRI	Ethiopia	Dr Fitsum Girma									
UND	USA	Dr Christian Koepfli									

LSHTM: London School of Hygiene and Tropical Medicine
UNC: University of North Carolina
ADFMIDI: Australian Defence Force Malaria and Infectious Disease Institute
CDC: Centres for Disease Control
UCAD: Université Cheikh Anta Diop de Dakar
UPCH: Universidad Peruana Cayetano Heredia
NIMR: National Institute of Malaria Research
AHRI: Amauer Hansen Research Institute
UND: University of Notre Dame

WHO EQA for malaria NAATs

- Began in Jan 2017
- 57 labs joined
- DBS and lyophilized whole blood with parasite densities: 0.05 - 2000 p/µL
- Distributed twice/pa
- Performance scored
- Gene deletion results reported
- More information: www.who.int/teams/global-malaria-programme/case-management/diagnosis/nucleic-acid-amplification-based-diagnostics/faq-nucleic-acid-amplification-tests

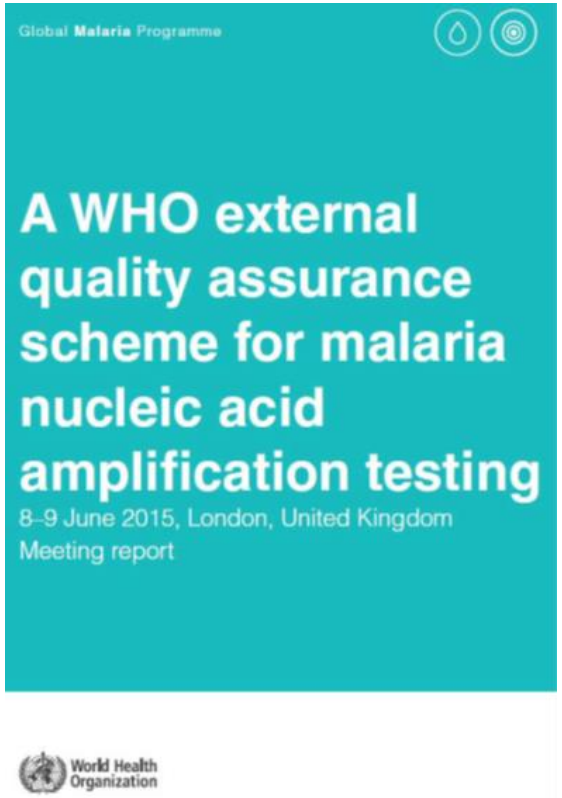

Table 3 Accuracy of external quality assessment results above and below a density of 2 parasites/µL

	Percentage of samples correctly identified[a]		*P* value
	≤ 2 parasites/µL	> 2 parasites/µL	
Species			
P. falciparum	54.8	91.9	< 0.01
P. vivax	86.9	92.5	0.32
P. knowlesi	69.2	69.1	0.98
P. malaria[b]	NA	48.7	NA
Sample type			
Dried blood spots	50.1	70.9	0.02
Lyophilized blood	80.5	85.8	0.18
Overall	68.3	79.1	0.03

[a] Results adjusted for laboratory capacity

[b] All samples of *P. malariae* were > 2 parasites/µL

Cunningham et al 2020 Malaria J

Join WHO NAAT EQA scheme: MalNAATEQA@who.int

CLOSING REMARKS

Community of Practice

***pfhrp2/3* gene deletions**

Mobilizing and providing peer and technical support

Available at MESA website:

www.mesamalaria.org

→ CoP **registration** through an online form

→ **MESA Resource compilation:**

Responding to the threat of pfhrp2/3 deletions