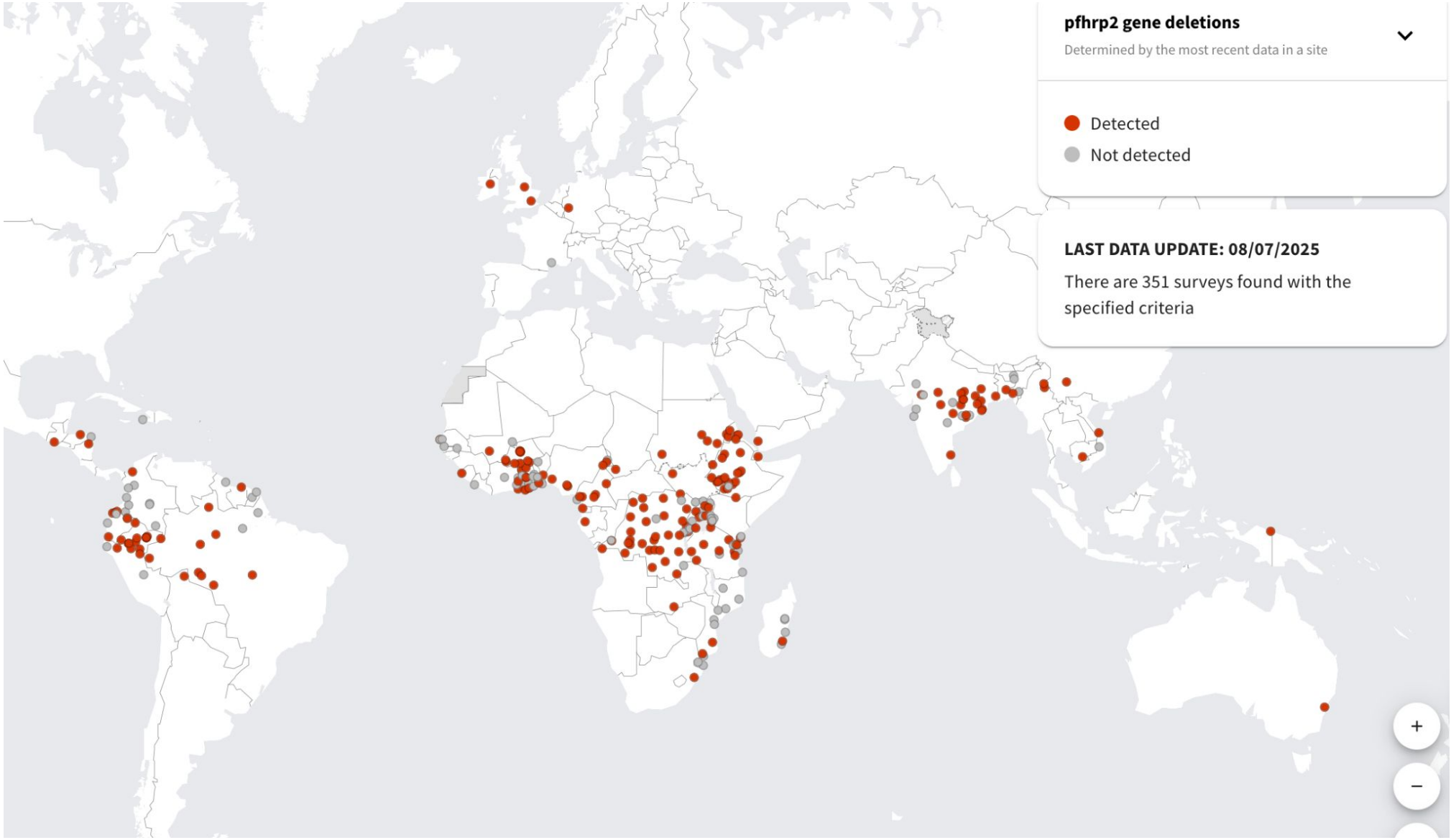
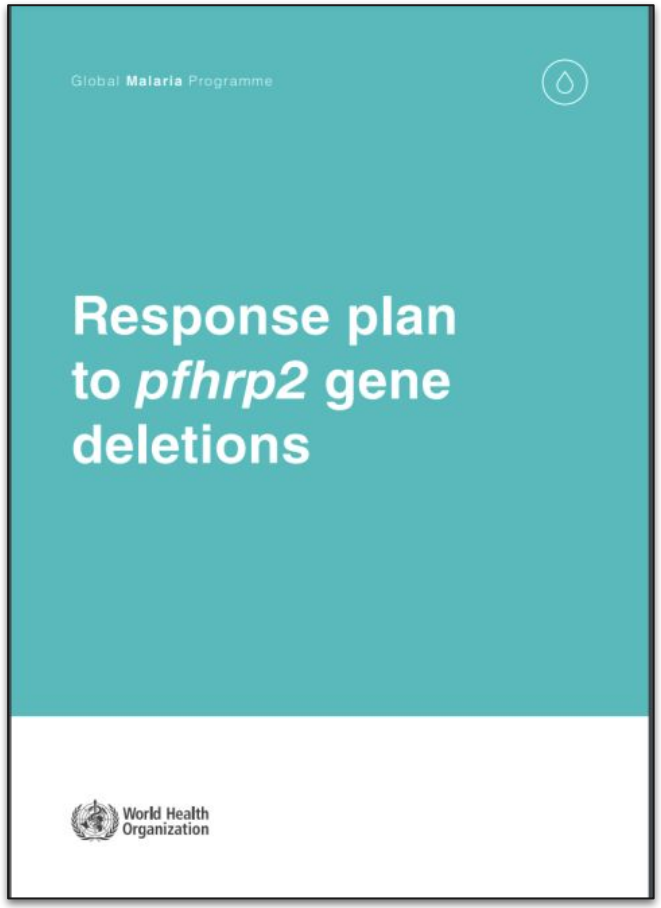
Malaria Molecular Surveillance Study Design Workshop

Module 6: The DRpower tool

Global landscape of *pfhrp2* deletions



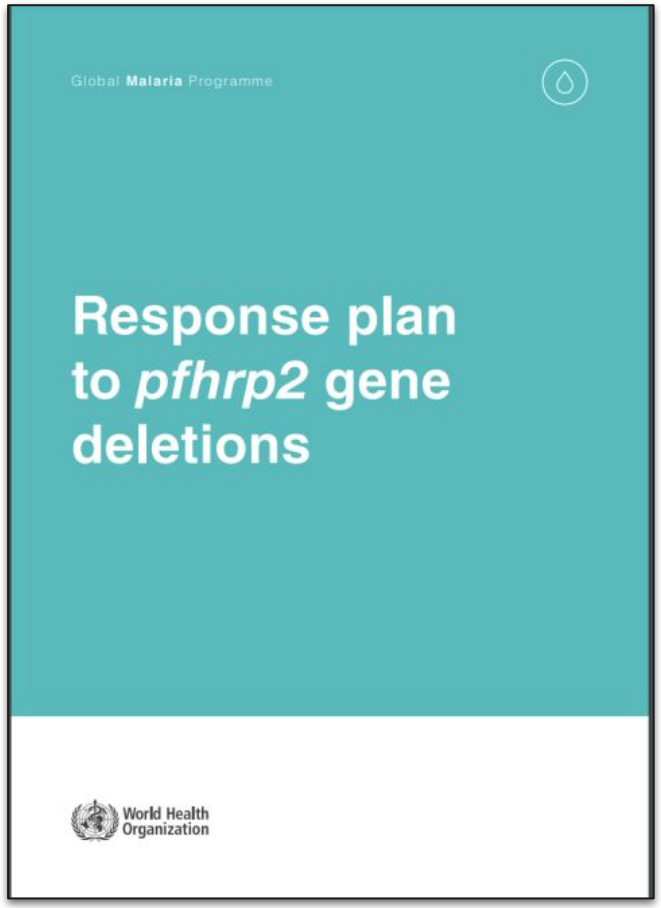
The WHO master protocol



Map the **distribution** and **frequency** of ***pfhrp2/3*** **deletion mutants**

Build an **international network of laboratories** to perform the complex **molecular confirmation** required for mapping and identify new and/or efficient screening methods

The WHO master protocol



Map the **distribution** and **frequency** of ***pfhrp2/3*** **deletion mutants**

Build an **international network of laboratories** to perform the complex **molecular confirmation** required for mapping and identify new and/or efficient screening methods

Standardized protocols for *P. falciparum* endemic countries to use to **determine prevalence of *pfhrp2/3* gene deletions causing false-negative RDTs**

Surveillance and **biobanking** protocols available

Each country will tailor protocol to their situation

The WHO master protocol

- Multi-cluster survey in a region with the following primary end-points:

1. Prevalence of **suspected false-negative HRP2 RDT** results among symptomatic patients with *P. falciparum* malaria

1. Prevalence of ***pfhrp2/3* gene deletions** among symptomatic falciparum patients with a false-negative HRP2 RDT result

1. Prevalence of ***pfhrp2/3* gene deletions causing false-negative HRP2 RDTs** among all symptomatic *P. falciparum* confirmed cases

The WHO master protocol

- WHO recommends **a nationwide change to non-HRP2-based RDTs** when the prevalence of clinically-significant pfhrp2/3 deletions (ie deletions causing false-negative RDT results) reaches 5% **in any region**

- Rationale: this is roughly the prevalence at which the *pfhrp2*-deleted infections detected by non-HRP2-based RDTs **would be outweighed by the reduced sensitivity of these RDTs**

- Literature review of published comparison data 2011–2022 showed the **5% threshold is still valid**

This is a **major policy change**, so it is crucial we get our study design right!

When to suspect *pfhrp2/3* deletions?

In a patient

Negative results on an HRP2 test line of at least two quality-assured malaria RDTs

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AND

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AND

Positive gold-standard test:

- Positive on the pan- or pf-pLDH test line, when a combination test is used
- Sample is confirmed microscopically to be positive for *P. falciparum* by two qualified microscopists

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Positive gold-standard test:

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

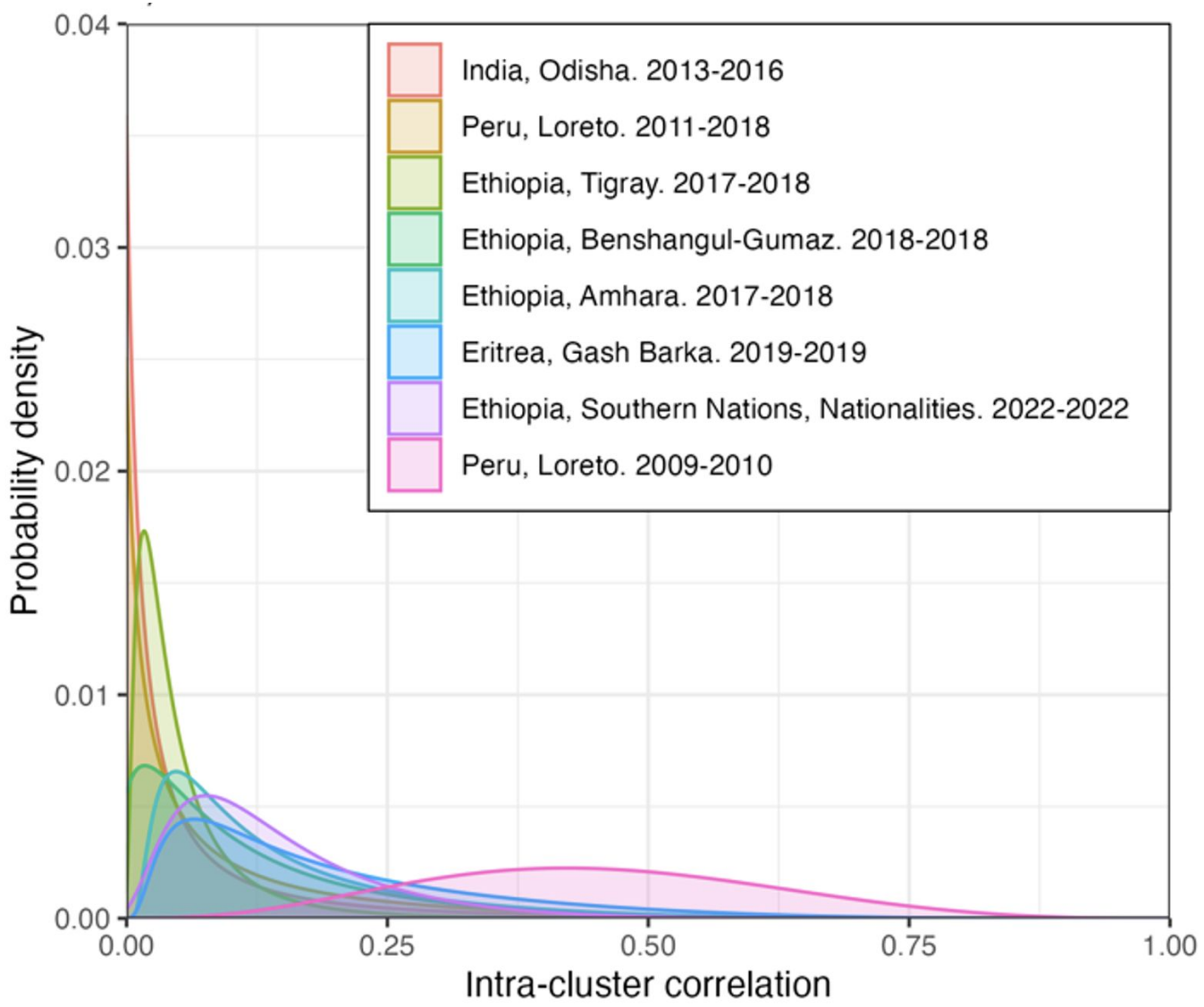
STATISTICAL ANALYSIS FRAMEWORK

- Bayesian framework comparing between two alternative hypotheses

- Estimate intra-cluster correlation automatically (incorporate *prior* information)

- Outputs:
  1. Maximum *a posteriori* (MAP) estimate of prevalence ("point estimate") with 95% Credible Interval (CrI)
  2. Posterior probability that prevalence is > 5% threshold
     - "Binary" test like traditional approach, but with advantages of Bayesian method

Historical analysis of ICC



The *updated* WHO master protocol

MRC Centre for Global Infectious Disease Analysis IMPERIAL

STATISTICAL ANALYSIS FRAMEWORK

1

Estimate prevalence and 95% credible interval (CrI)

The *updated* WHO master protocol

STATISTICAL ANALYSIS FRAMEWORK

1

Estimate prevalence and 95% credible interval (CrI)

2

Estimate the **probability** that the prevalence of *pfhrp2/3* deletions is above 5%

The *updated* WHO master protocol

STATISTICAL ANALYSIS FRAMEWORK

1

Estimate prevalence and 95% credible interval (CrI)

2

Estimate the **probability** that the prevalence of *pfhrp2/3* deletions is above 5%

Posterior probability is **less than or equal to 0.95**

OUTCOME 1

There is **not sufficient evidence** to conclude that the proportion of parasites with *pfhrp2/3* deletions causing false-negative HRP2 RDT results within symptomatic patients is greater than 5%

The *updated* WHO master protocol

MRC Centre for Global Infectious Disease Analysis | IMPERIAL

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OUTCOME 1

There is **not sufficient evidence** to conclude that the proportion of parasites with *pfhrp2/3* deletions causing false-negative HRP2 RDT results within symptomatic patients is greater than 5%

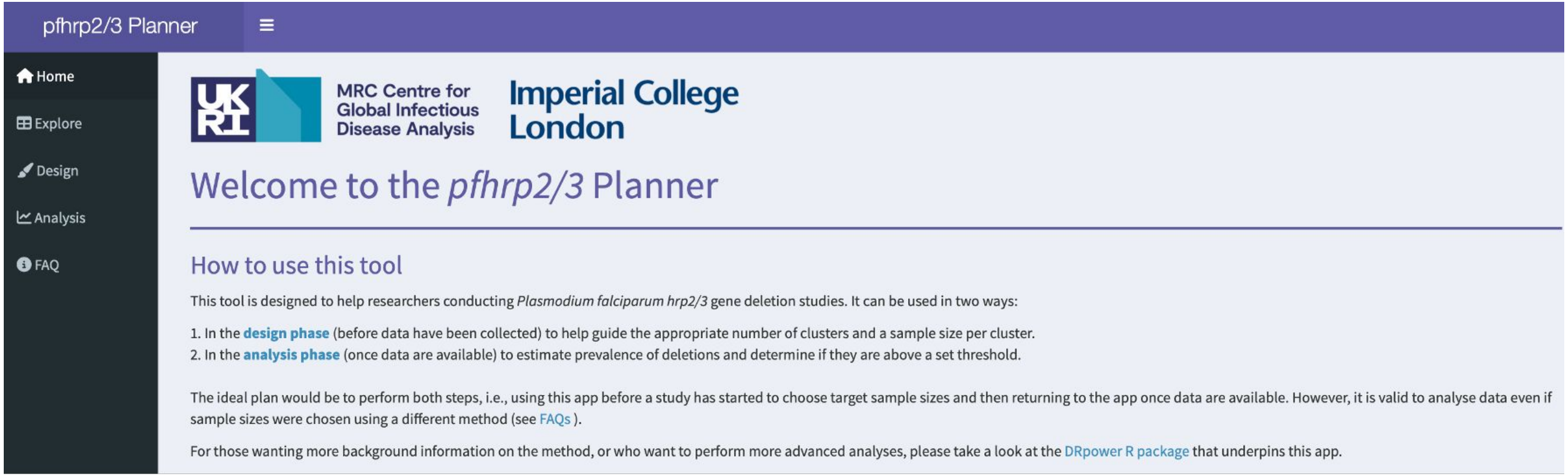
Posterior probability is **greater than 0.95**

OUTCOME 2

There is **high statistical confidence** that the proportion of parasites with *pfhrp2/3* deletions causing false-negative HRP2 RDT results within symptomatic patients is greater than 5%

The DRpower *pfhrp2/3* planner tool

- Easy-to-use web interface, uses the DRpower R package in the back-end
- End-users can: design their study and/or analyse study results



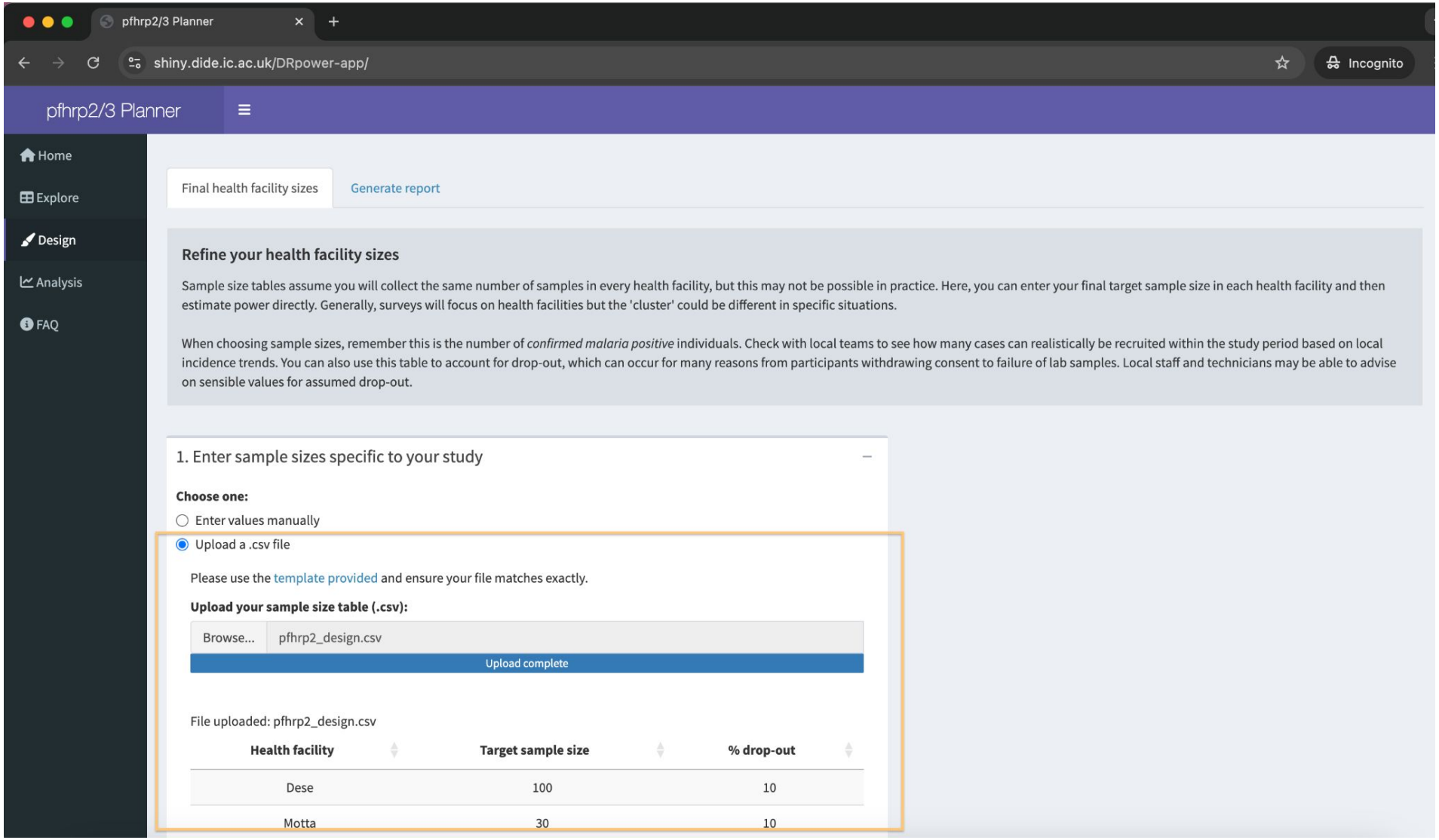
Explore sample sizes

- How many samples? How many health facilities (or clusters)?
  - DRpower includes pre-computed sample sizes per health facility to achieve 80% power

Number of health facilities	1%	2%	3%	4%	5%	6%	7%	8%	9	10%	11%	12%	13%	14%	15%	16%	17%	18%	19%	20%
2													344	140	62	38	33	22	18	14
3												172	69	41	26	20	16	14	12	9
4											128	60	33	22	16	13	10	9	8	7
5										**496**	75	36	22	16	10	7	7	5	5	5
6										**113**	47	25	16	12	9	6	5	5	5	5
7										**68**	30	18	13	10	7	6	5	5	5	5
8									416	**51**	23	15	10	9	7	5	5	5	5	5
9									138	**37**	20	13	10	8	6	5	5	5	5	5
10									85	**30**	15	12	8	6	5	5	5	5	5	5

Blank = >2000

Design your study



Downloadable reports

MRC Centre for Global Infectious Disease Analysis | IMPERIAL

pfhrp2/3 Planner: Design report

Downloaded on: 2024-07-02 | DRpower interactive app v1.0.1

Background

This report presents the results generated by using the *pfhrp2/3* planner web application. This application was used to help guide the appropriate number of health facilities (or sites) and a target sample size per health facility for the design of a *pfhrp2/3* deletion prevalence study. For more information on the statistical method used, see the DRpower R package website.

Final sample sizes

The adjusted sample sizes based on the expected drop-out proportion in each of the 3 health facilities are shown below and should be considered for study design.

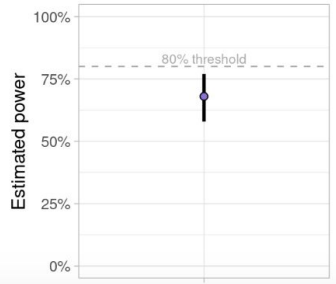
Health facility	Target sample size	Dropout (%)	Adjusted sample size
1	500	10	556
2	500	10	556
3	500	10	556
4	100	3	104
Total samples	*1600*	–	*1772*

Estimated power

To estimate the power of the study assuming the target sample sizes as per above, we used the DRpower R package with the following parameters:

- Prevalence of 10%
- Intra-cluster correlation of 0.05
- 100 simulations

The estimated power is 68% (95%CI: 58 - 77%).



Analyze your results

Prevalence estimates

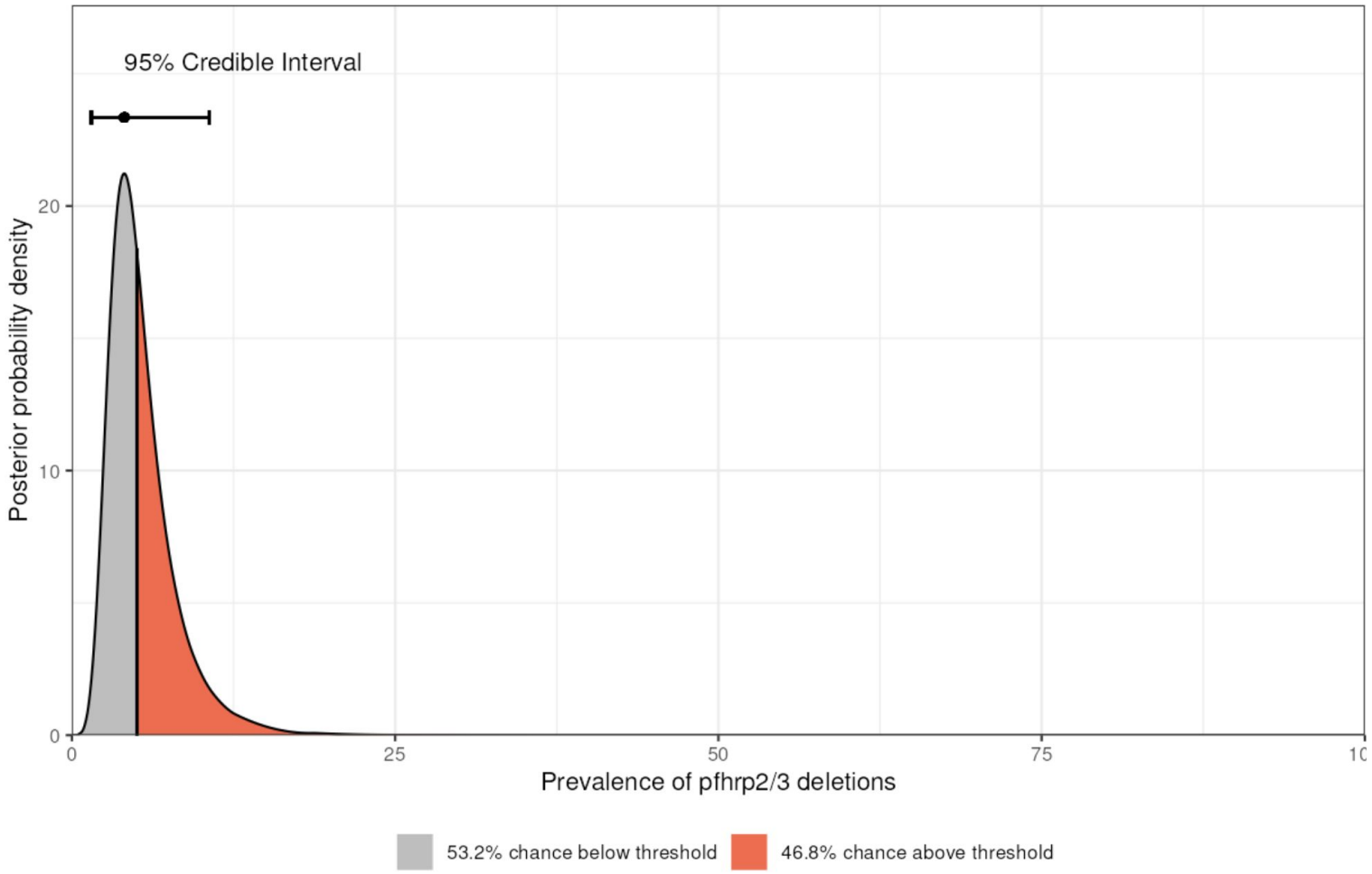
The table and the plot below show the maximum a posteriori (MAP) estimate of the prevalence, along with a 95% credible interval (CrI). The MAP estimate can be used as a central estimate of the prevalence, but it should always be reported alongside the CrI to give a measure of uncertainty.

Prevalence estimate (%)	Lower CrI (%)	Upper CrI (%)	Probability above threshold (%)
4.05	1.50	10.61	46.75

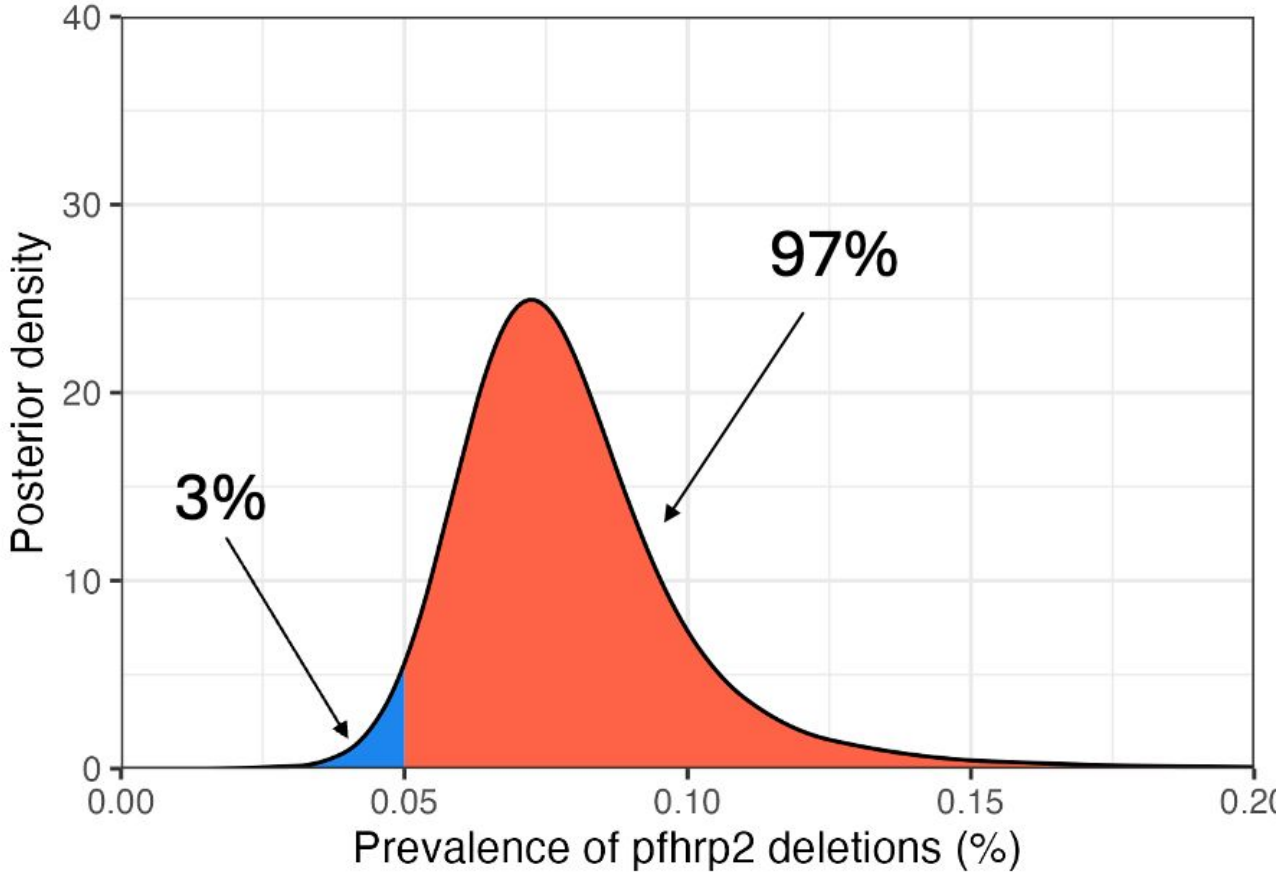
RESULT: We estimate that the prevalence of *pfhrp2/3* deletions is 4.05 % (95% CrI: 1.5 - 10.61 %). The probability that the prevalence is above the 5% threshold is estimated at 46.75 %. *We require greater than 95% probability to confidently conclude that prevalence is above the 5% threshold.*

CONCLUSION: The prevalence of *pfhrp2/3* deletions causing false-negative HRP2 RDTs is **below** the 5% threshold.

Analyze your results



Analyze your results



Downloadable reports

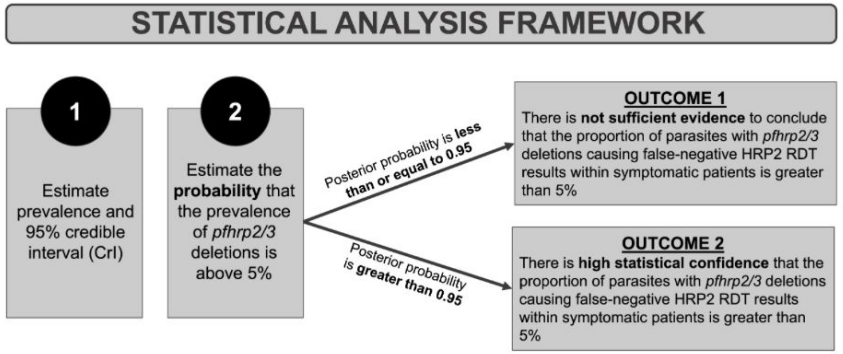
pfhrp2/3 Planner: Analysis report

Downloaded on: 2024-07-02 | DRpower interactive app v1.0.1

Background

This report presents the results generated by the *pfhrp2/3* planner web application. This application was used to analyze data from a *pfhrp2/3* deletion prevalence study to estimate prevalence and determine if it is above the WHO recommended prevalence threshold of 5%. This software uses a Bayesian hierarchical model implemented in the DRpower R package to estimate the prevalence of *pfhrp2/3* deletions causing false-negative HRP2 RDT results while accounting for correlations within clusters or health facilities/sites. For more information on the statistical method used, see the DRpower R package website.

The statistical analysis framework involves calculation of a point estimate of the prevalence of deletions along with a 95% credible interval (CrI) to summarise results, with the probability that the prevalence of *pfhrp2/3* deletions is above 5% used to categorize study domains into outcome 1 or 2, as follows:



Study details

A total of 8 health facilities (or sites) were surveyed in this *pfhrp2/3* deletion prevalence study. The observed counts of *pfhrp2/3* deletions causing false-negative HRP2 RDTs and the sample sizes in each health facility are shown below:

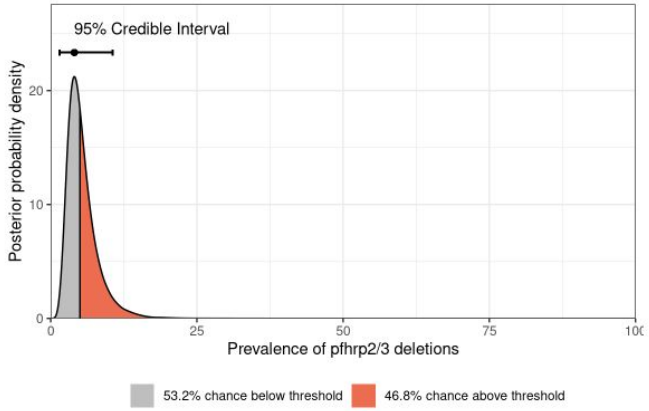
Health facility	Number of deletions	Sample size
Northwestern Health Facility	0	60
Northern Health Facility	0	60
Western Health Facility	1	60
Eastern Health Facility	2	60
Central Health Facility	5	60
Southern Health Facility	5	51
Southeastern Health Facility	3	51
Community Health Facility	0	20

Estimated prevalence

To estimate the prevalence of *pfhrp2/3* deletions causing false-negative HRP2 RDTs, we used to test whether the observed prevalence in this study was above or below the

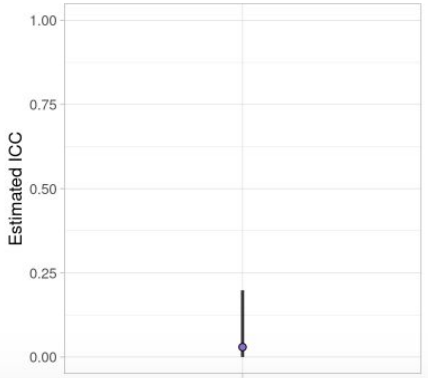
RESULT: We estimate that the prevalence of *pfhrp2/3* deletions causing false-negative HRP2 RDTs is 4.05% (95% CrI: 1.5-10.61%). The probability that the prevalence is above the 5% threshold is estimated at 46.75%. *We require greater than 95% probability to confidently conclude that prevalence is above the 5% threshold.*

CONCLUSION: The prevalence of *pfhrp2/3* deletions causing false-negative HRP2 RDTs within symptomatic *P. falciparum* patients is below the 5% WHO threshold.



Estimated intra-cluster correlation (ICC)

We estimate that the intra-cluster correlation is 0.03 (95% CrI: 0-0.2).



Summary

- Global priority to monitor *pfhrp2* gene deletion prevalence
- The updated WHO master protocol will recommend a new method based on a Bayesian framework
- This framework is more powerful, but more mathematically complex
- We developed the DRpower *pfhrp2/3* planner tool where users can design multi-cluster *pfhrp2/3* surveys, and also analyze results once collected
- The DRpower R package also has functionality for more than just *pfhrp2/3* deletion surveys (eg detection of rare variants, sample size based on MOE)

Module 6 activity

Format: Interactive R code, accessed through the web, alongside use of the web-based *pfhrp2/3* Planner

- How to design a multi-cluster *pfhrp2/3* deletion study.
- How to analyse and interpret the results of a *pfhrp2/3* deletion study.
- How to account for intra-cluster correlation in other study designs, such as prevalence surveys and presence/absence studies.

Workshop materials

https://mrc-ide.github.io/MMS-SD_workshop/