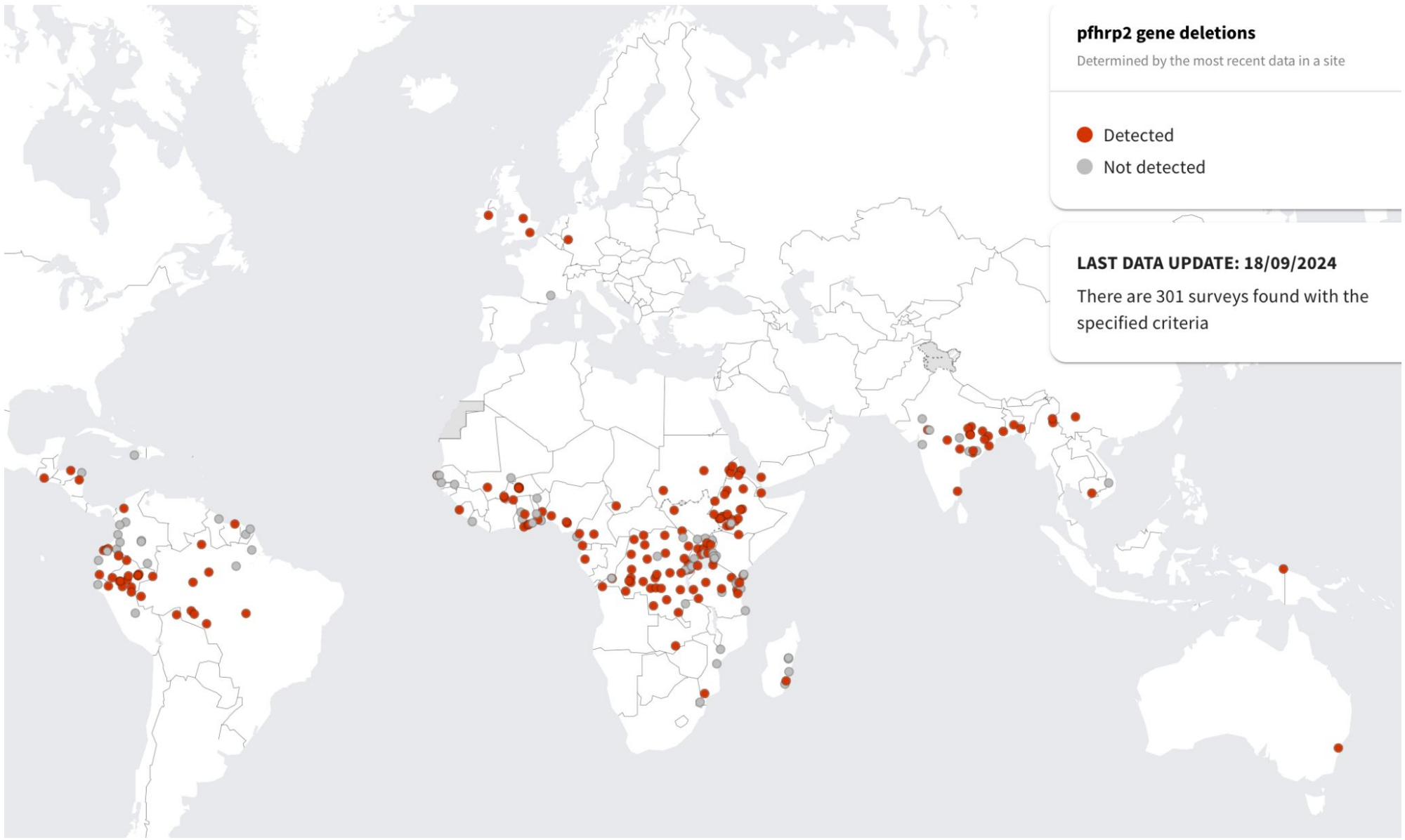
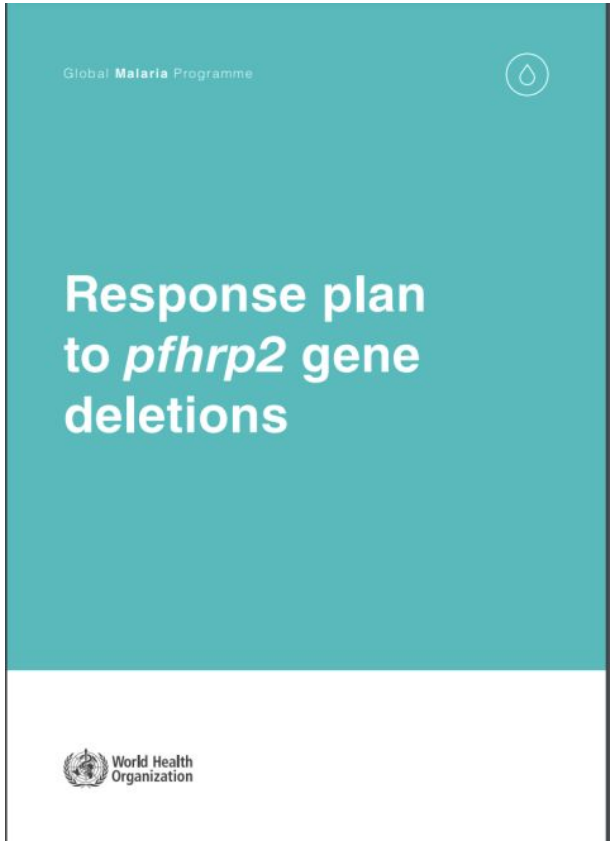
# Malaria Molecular Surveillance Study Design Workshop

## **Module 5**: The DRpower tool

# Global landscape of *pfhrp2* deletions



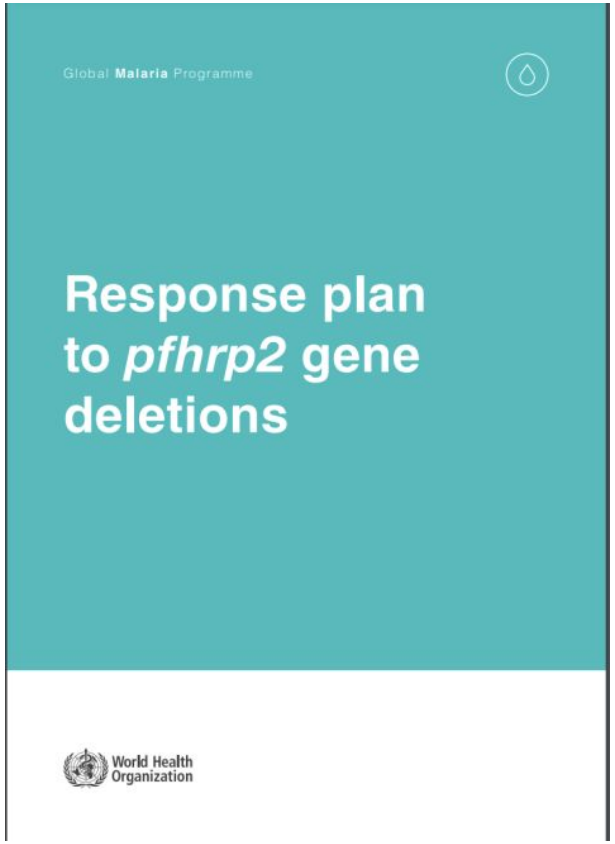
# The WHO master protocol




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

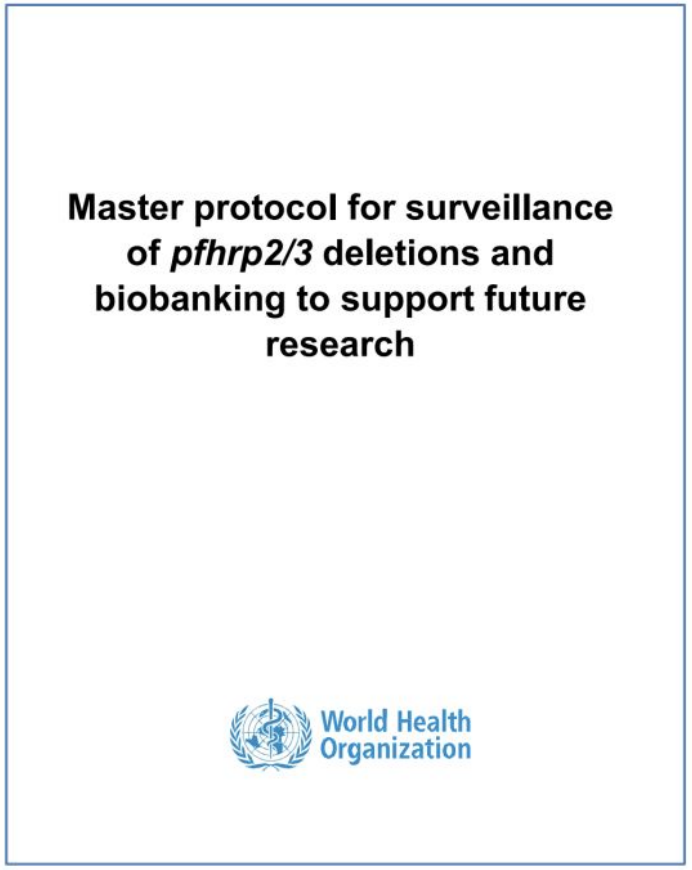
Build an **international network of laboratories** to perform the complex **molecular confirmation** required for mapping and identify new and/or efficient screening methods

# 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

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

3. 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?

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In a patient

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

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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

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- Positive on the pan- or pf-pLDH test line, when a combination test is used
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*Also consider travel history to areas with high prevalence of HRP2 deletions e.g. Peru, Brazil, Eritrea, Djibouti, Ethiopia*

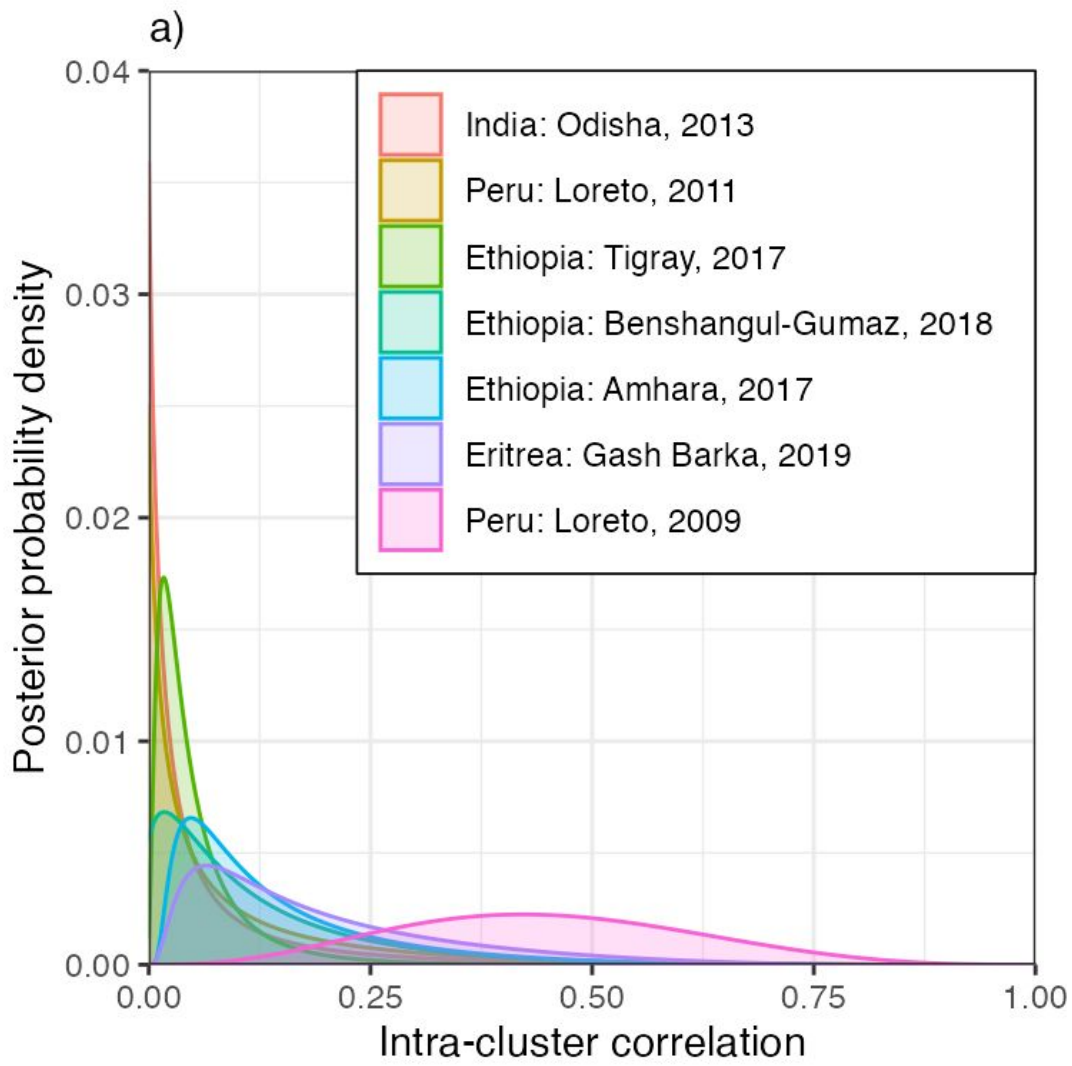
# STATISTICAL ANALYSIS FRAMEWORK

- 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

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## 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

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Estimate prevalence and 95% credible interval (CrI)

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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%

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## STATISTICAL ANALYSIS FRAMEWORK

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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%

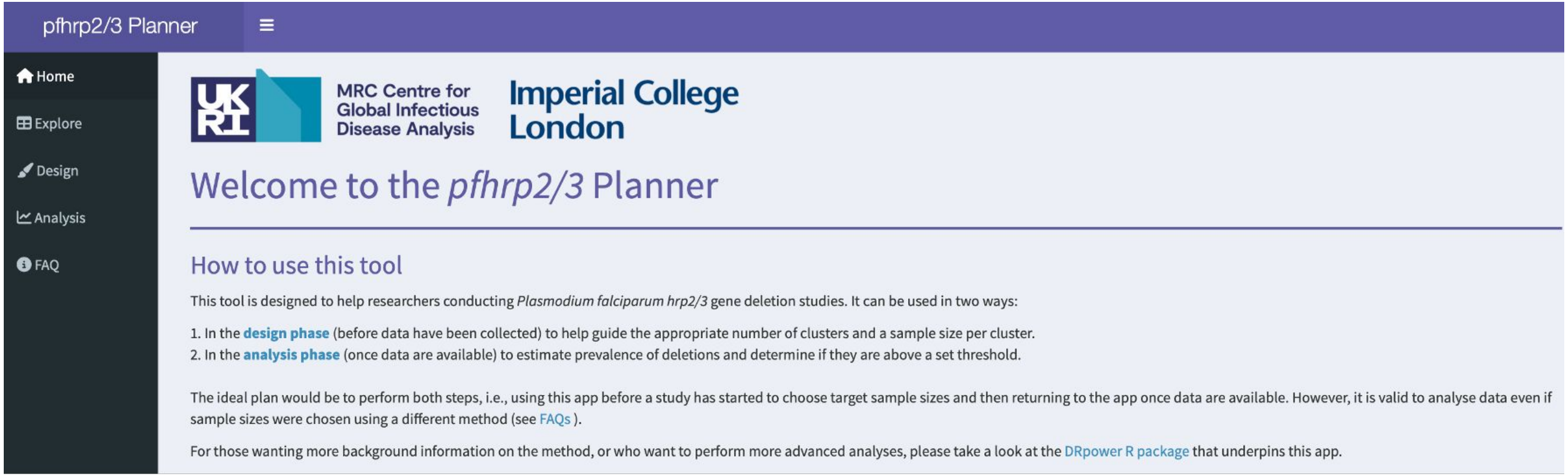
Posterior probability is **greater than 0.95**

**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



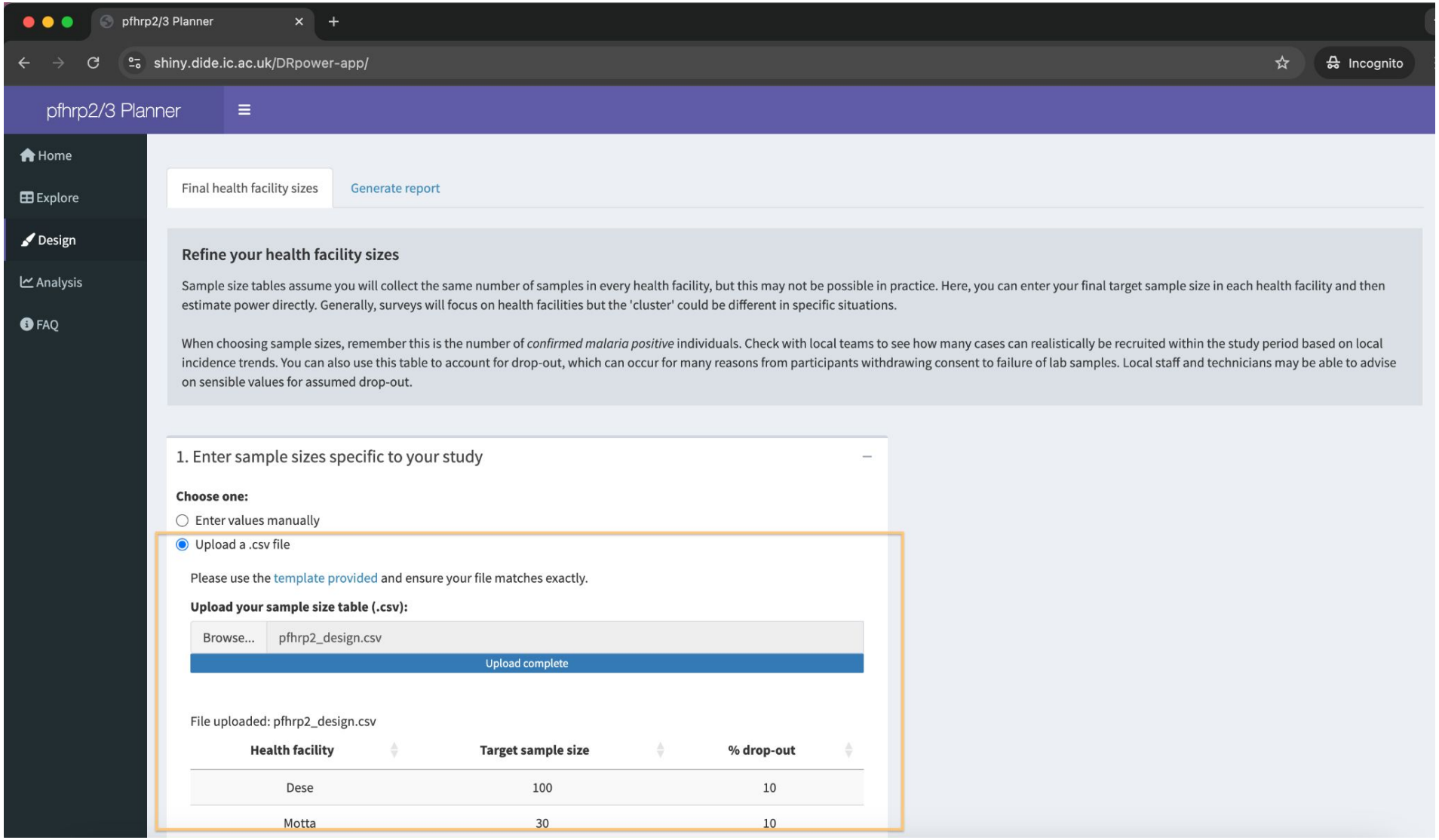
# Explore sample sizes

- 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

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## *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.

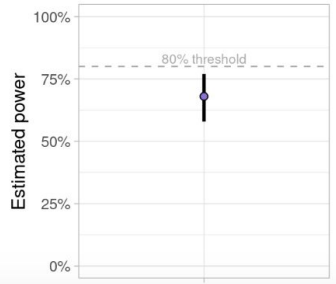
| Health facility | Target sample size | Dropout (%) | Adjusted sample size |
|---|---|---|---|
| 1 | 500 | 10 | 556 |
| 2 | 500 | 10 | 556 |
| 3 | 500 | 10 | 556 |
| 4 | 100 | 3 | 104 |
| *Total samples* | *1600* | – | *1772* |

### 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

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## Prevalence estimates

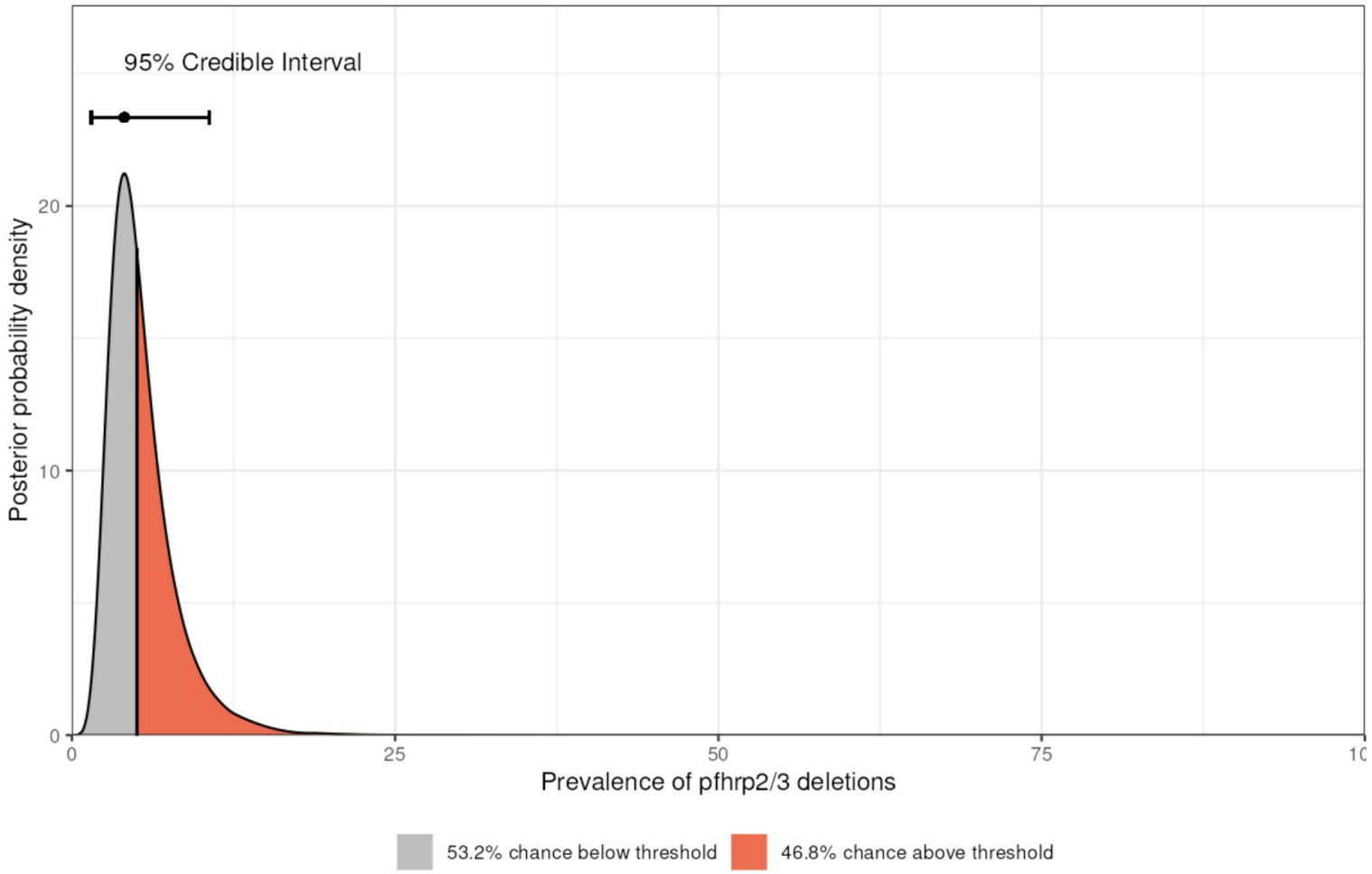
The table and the plot below show the maximum a posteriori (MAP) estimate of the prevalence, along with a 95% credible interval (CrI). The MAP estimate can be used as a central estimate of the prevalence, but it should always be reported alongside the CrI to give a measure of uncertainty.

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

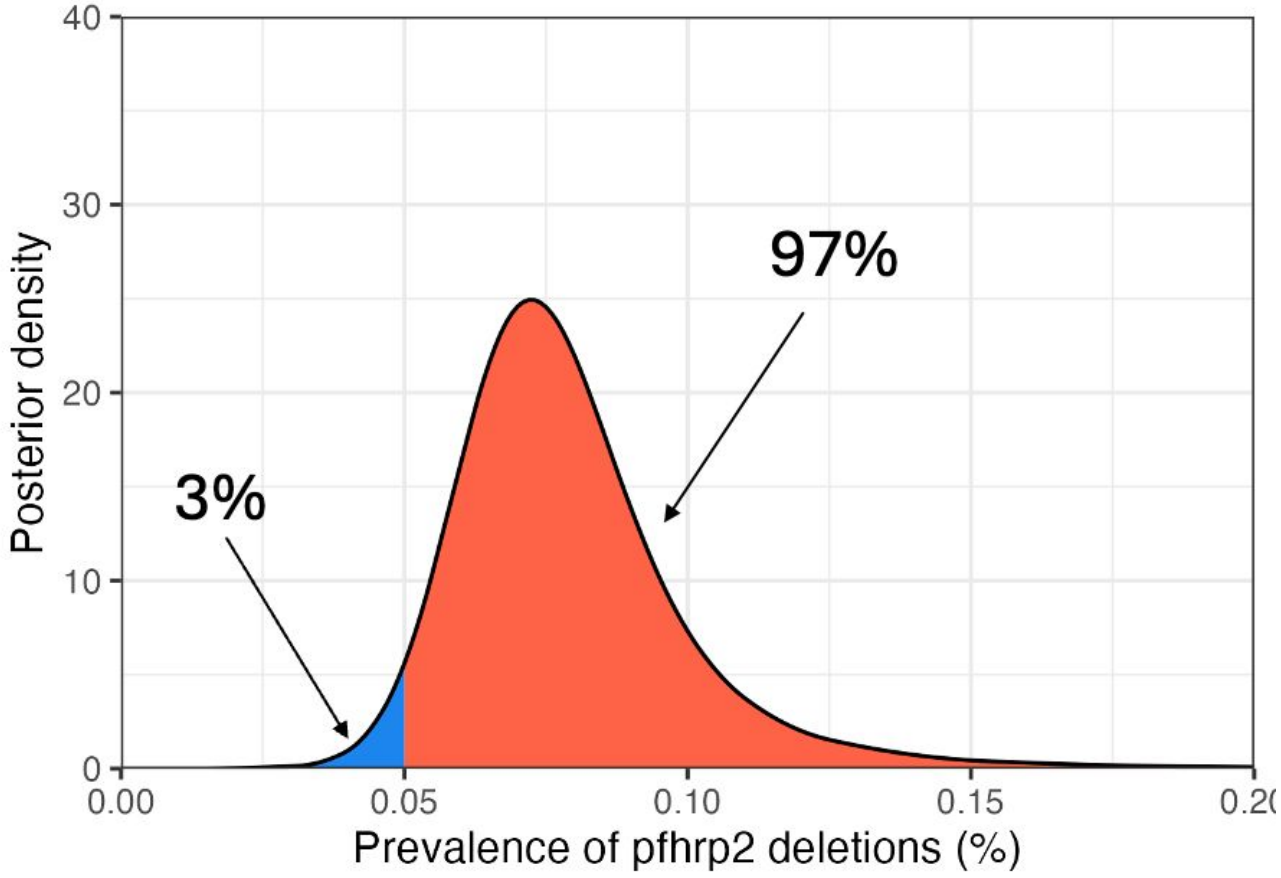
RESULT: We estimate that the prevalence of *pfhrp2/3* deletions is 4.05 % (95% CrI: 1.5 - 10.61 %). The probability that the prevalence is above the 5% threshold is estimated at 46.75 %. *We require greater than 95% probability to confidently conclude that prevalence is above the 5% threshold.*

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

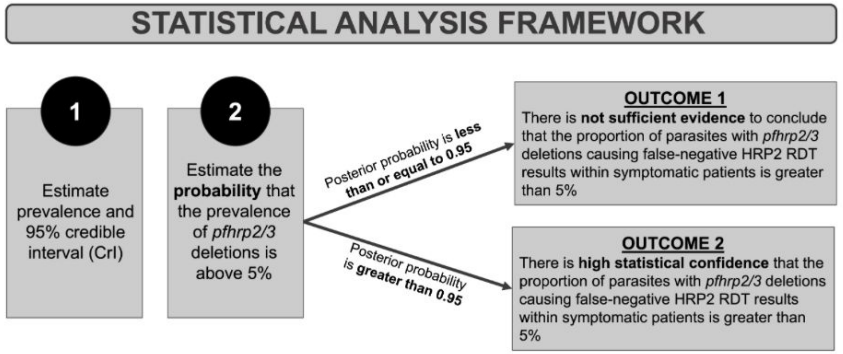
## *pfhrp2/3* Planner: Analysis report

*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:

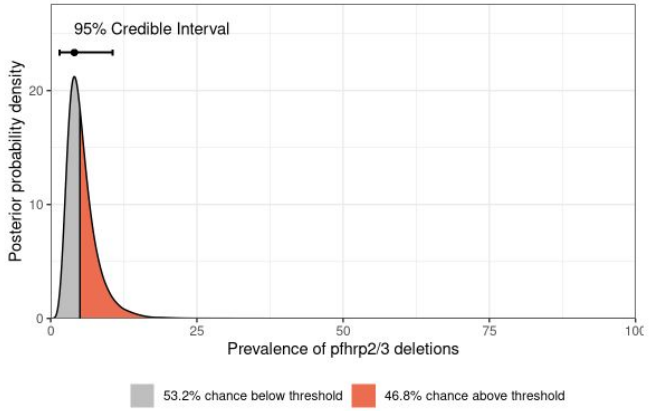
| Health facility | Number of deletions | Sample size |
|---|---|---|
| Northwestern Health Facility | 0 | 60 |
| Northern Health Facility | 0 | 60 |
| Western Health Facility | 1 | 60 |
| Eastern Health Facility | 2 | 60 |
| Central Health Facility | 5 | 60 |
| Southern Health Facility | 5 | 51 |
| 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

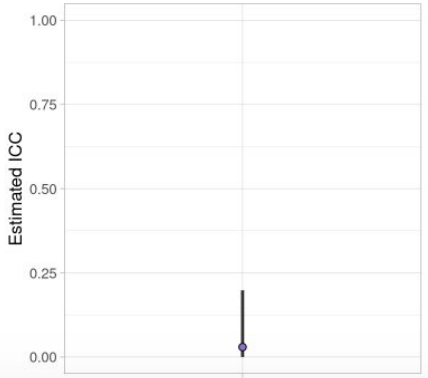
**RESULT**: We estimate that the prevalence of *pfhrp2/3* deletions causing false-negative HRP2 RDTs is 4.05% (95% CrI: 1.5-10.61%). The probability that the prevalence is above the 5% threshold is estimated at 46.75%. *We require greater than 95% probability to confidently conclude that prevalence is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