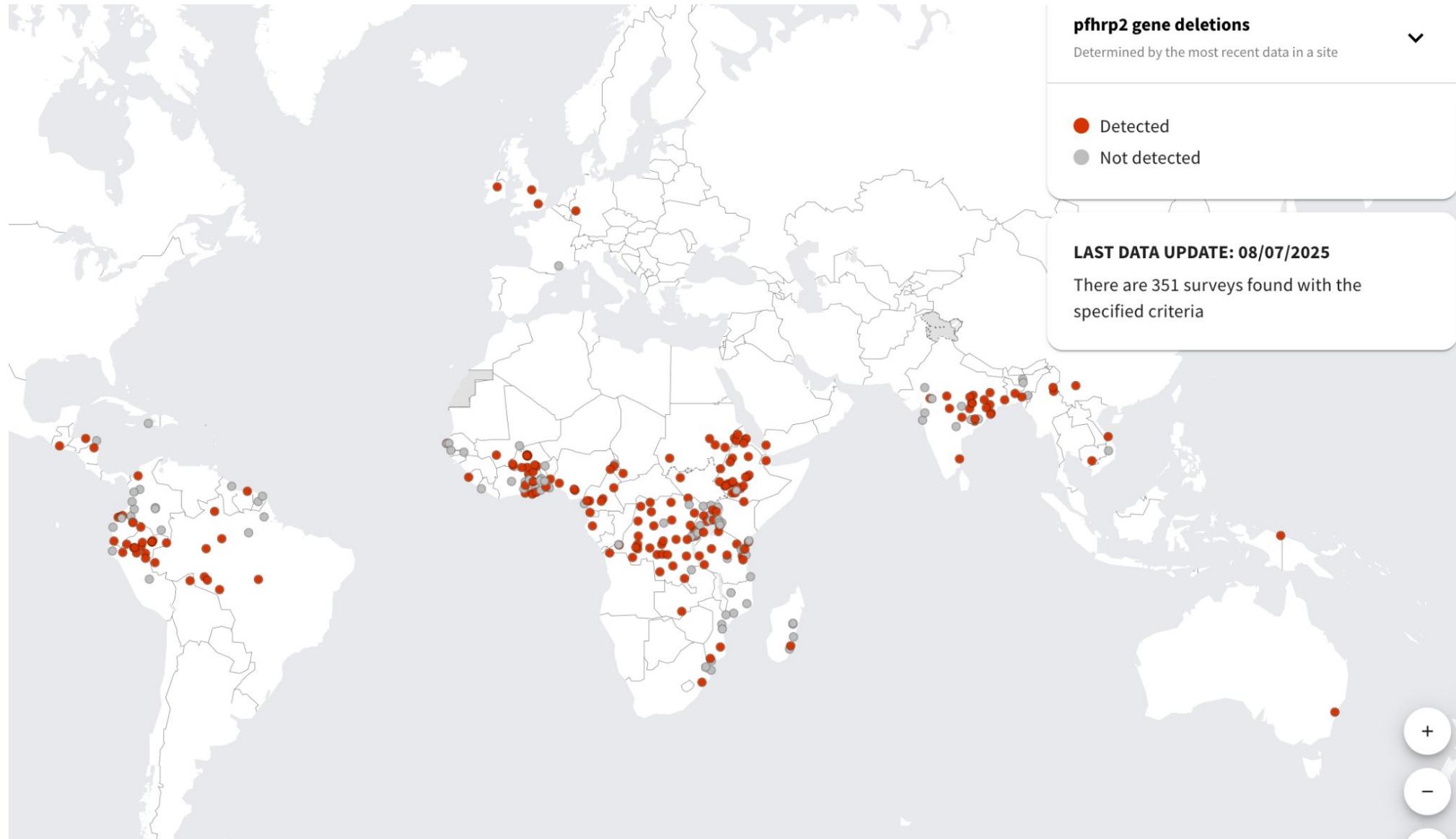


# Malaria Molecular Surveillance Study Design Workshop

## Module 6: The DRpower tool

# Global landscape of *pfhrp2* deletions



Global Malaria Programme



## Response plan to *pfhrp2* gene deletions



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

Global Malaria Programme



## Response plan to *pfhrp2* gene deletions



Map the **distribution** and  
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deletion mutants

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

**Master protocol for  
surveillance of *pfhrp2/3*  
deletions and biobanking  
to support future research**

Second edition



**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

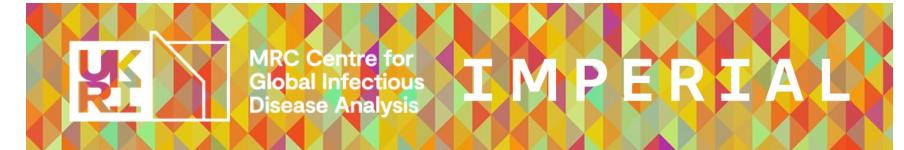
**Each country will tailor  
protocol to their  
situation**

- 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

- 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!

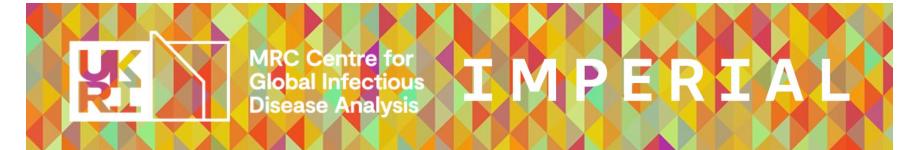
# When to suspect *pfhrp2/3* deletions?



## In a patient

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

# When to suspect *pfhrp2/3* deletions?



In a patient

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

AND

## In a patient

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

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

## In a patient

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

AND

### **Positive gold-standard test:**

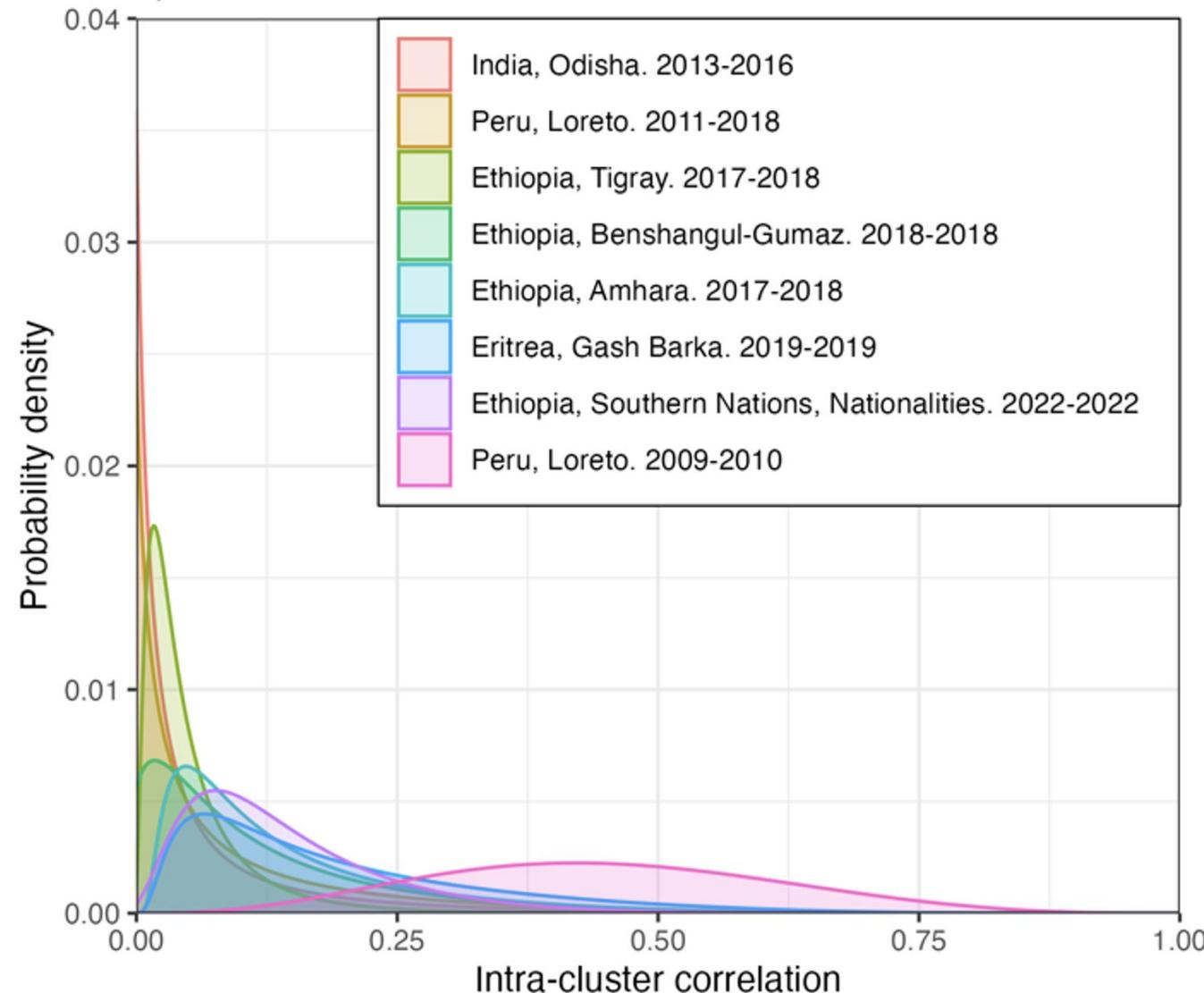
- Positive on the pan- or pf-pLDH test line, when a combination test is used
- Sample is confirmed microscopically to be positive for *P. falciparum* by two qualified microscopists

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 (Crl)
  2. Posterior probability that prevalence is  $> 5\%$  threshold
    - “Binary” test like traditional approach, but with advantages of Bayesian method

# Historical analysis of ICC



## STATISTICAL ANALYSIS FRAMEWORK

1

Estimate  
prevalence and  
95% credible  
interval (CrI)

## 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%

## 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%

## 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

Posterior probability is greater than 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%

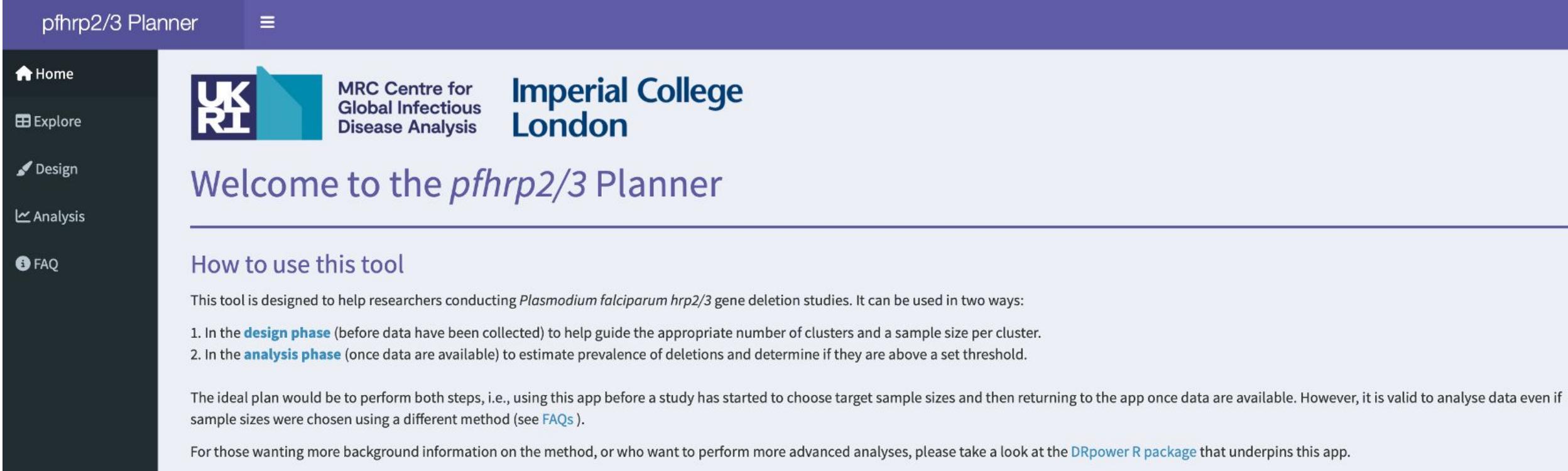
### **OUTCOME 2**

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



The screenshot shows the 'pfhrp2/3 Planner' web application. The top navigation bar is purple with the title 'pfhrp2/3 Planner' on the left and a menu icon on the right. A vertical sidebar on the left contains icons for 'Home' (house), 'Explore' (grid), 'Design' (pencil), 'Analysis' (chart), and 'FAQ' (info). The main content area has a dark blue header with the UKRI logo, the text 'MRC Centre for Global Infectious Disease Analysis', and 'Imperial College London'. Below this is a large purple header with the text 'Welcome to the pfhrp2/3 Planner'. The main content area starts with a section titled 'How to use this tool' with the following text: 'This tool is designed to help researchers conducting *Plasmodium falciparum* *hrp2/3* gene deletion studies. It can be used in two ways: 1. In the [design phase](#) (before data have been collected) to help guide the appropriate number of clusters and a sample size per cluster. 2. In the [analysis phase](#) (once data are available) to estimate prevalence of deletions and determine if they are above a set threshold.' It then continues with: 'The ideal plan would be to perform both steps, i.e., using this app before a study has started to choose target sample sizes and then returning to the app once data are available. However, it is valid to analyse data even if sample sizes were chosen using a different method (see [FAQs](#)).' At the bottom, it says: 'For those wanting more background information on the method, or who want to perform more advanced analyses, please take a look at the [DRpower R package](#) that underpins this app.'

pfhrp2/3 Planner

Home

Explore

Design

Analysis

FAQ

UKRI

MRC Centre for Global Infectious Disease Analysis

Imperial College London

## Welcome to the pfhrp2/3 Planner

### How to use this tool

This tool is designed to help researchers conducting *Plasmodium falciparum* *hrp2/3* gene deletion studies. It can be used in two ways:

1. In the [design phase](#) (before data have been collected) to help guide the appropriate number of clusters and a sample size per cluster.
2. In the [analysis phase](#) (once data are available) to estimate prevalence of deletions and determine if they are above a set threshold.

The ideal plan would be to perform both steps, i.e., using this app before a study has started to choose target sample sizes and then returning to the app once data are available. However, it is valid to analyse data even if sample sizes were chosen using a different method (see [FAQs](#)).

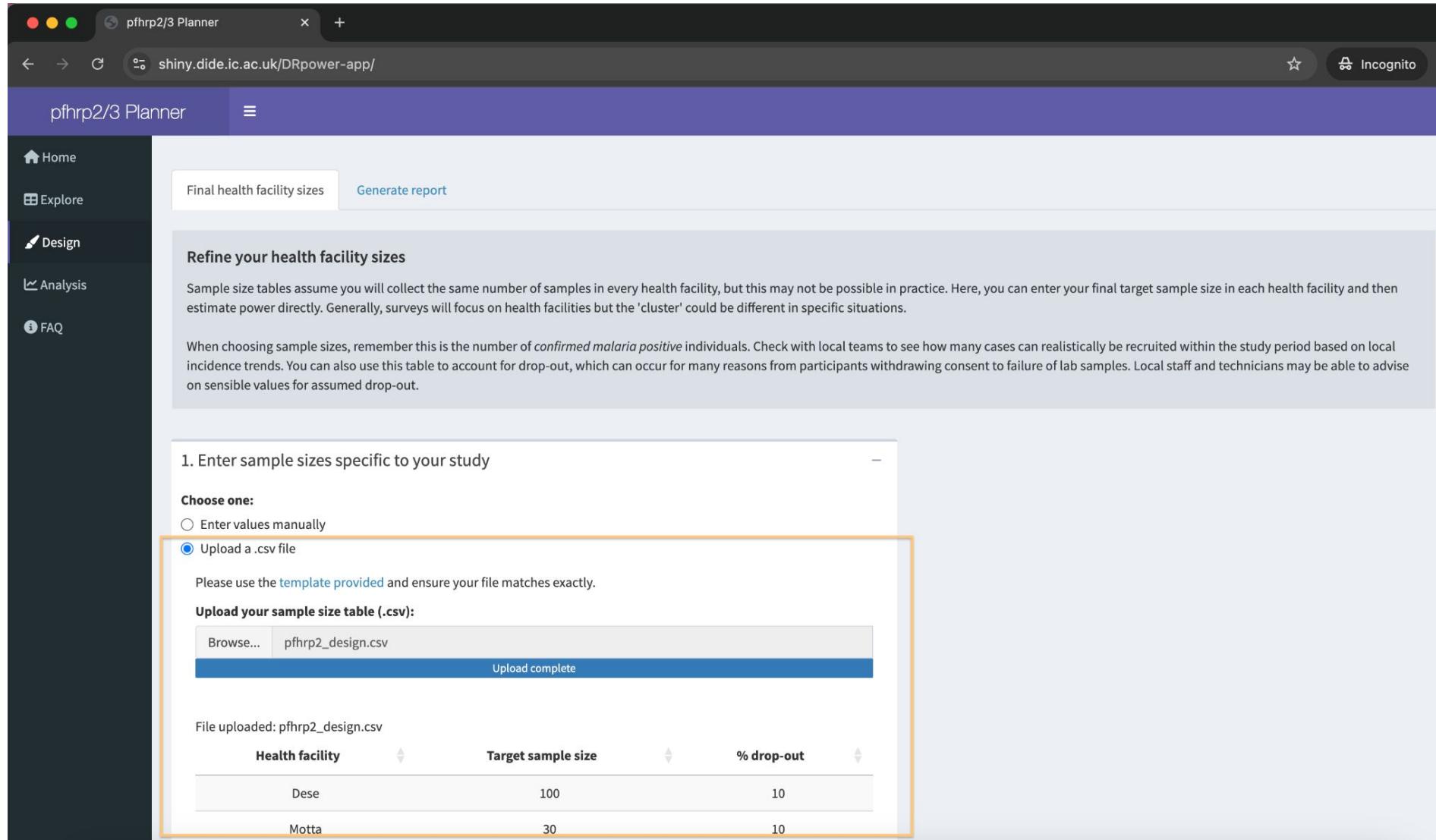
For those wanting more background information on the method, or who want to perform more advanced analyses, please take a look at the [DRpower R package](#) that underpins this app.

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

| Number<br>of<br>health<br>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                                    |    |    |    |    |    |    |    |     |           | <b>496</b> | 75  | 36  | 22  | 16  | 10  | 7   | 7   | 5   | 5   | 5   |
| 6                                    |    |    |    |    |    |    |    |     |           | <b>113</b> | 47  | 25  | 16  | 12  | 9   | 6   | 5   | 5   | 5   | 5   |
| 7                                    |    |    |    |    |    |    |    |     |           | <b>68</b>  | 30  | 18  | 13  | 10  | 7   | 6   | 5   | 5   | 5   | 5   |
| 8                                    |    |    |    |    |    |    |    | 416 | <b>51</b> | 23         | 15  | 10  | 9   | 7   | 5   | 5   | 5   | 5   | 5   |     |
| 9                                    |    |    |    |    |    |    |    | 138 | <b>37</b> | 20         | 13  | 10  | 8   | 6   | 5   | 5   | 5   | 5   | 5   |     |
| 10                                   |    |    |    |    |    |    |    | 85  | <b>30</b> | 15         | 12  | 8   | 6   | 5   | 5   | 5   | 5   | 5   | 5   |     |

Blank = >2000

# Design your study



pfhrp2/3 Planner

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

pfhrp2/3 Planner

Home

Explore

Design

Analysis

FAQ

Final health facility sizes

Generate report

Refine your health facility sizes

Sample size tables assume you will collect the same number of samples in every health facility, but this may not be possible in practice. Here, you can enter your final target sample size in each health facility and then estimate power directly. Generally, surveys will focus on health facilities but the 'cluster' could be different in specific situations.

When choosing sample sizes, remember this is the number of *confirmed malaria positive* individuals. Check with local teams to see how many cases can realistically be recruited within the study period based on local incidence trends. You can also use this table to account for drop-out, which can occur for many reasons from participants withdrawing consent to failure of lab samples. Local staff and technicians may be able to advise on sensible values for assumed drop-out.

1. Enter sample sizes specific to your study

Choose one:

Enter values manually

Upload a .csv file

Please use the [template provided](#) and ensure your file matches exactly.

Upload your sample size table (.csv):

Browse... pfhrp2\_design.csv

Upload complete

File uploaded: pfhrp2\_design.csv

| Health facility | Target sample size | % drop-out |
|-----------------|--------------------|------------|
| Dese            | 100                | 10         |
| Motta           | 30                 | 10         |

## 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.

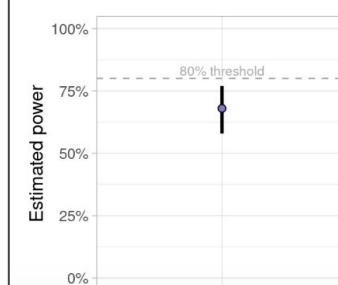
| Health facility      | Target sample size | Dropout (%) | Adjusted sample size |
|----------------------|--------------------|-------------|----------------------|
| 1                    | 500                | 10          | 556                  |
| 2                    | 500                | 10          | 556                  |
| 3                    | 500                | 10          | 556                  |
| 4                    | 100                | 3           | 104                  |
| <i>Total samples</i> | 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%).



## Prevalence estimates

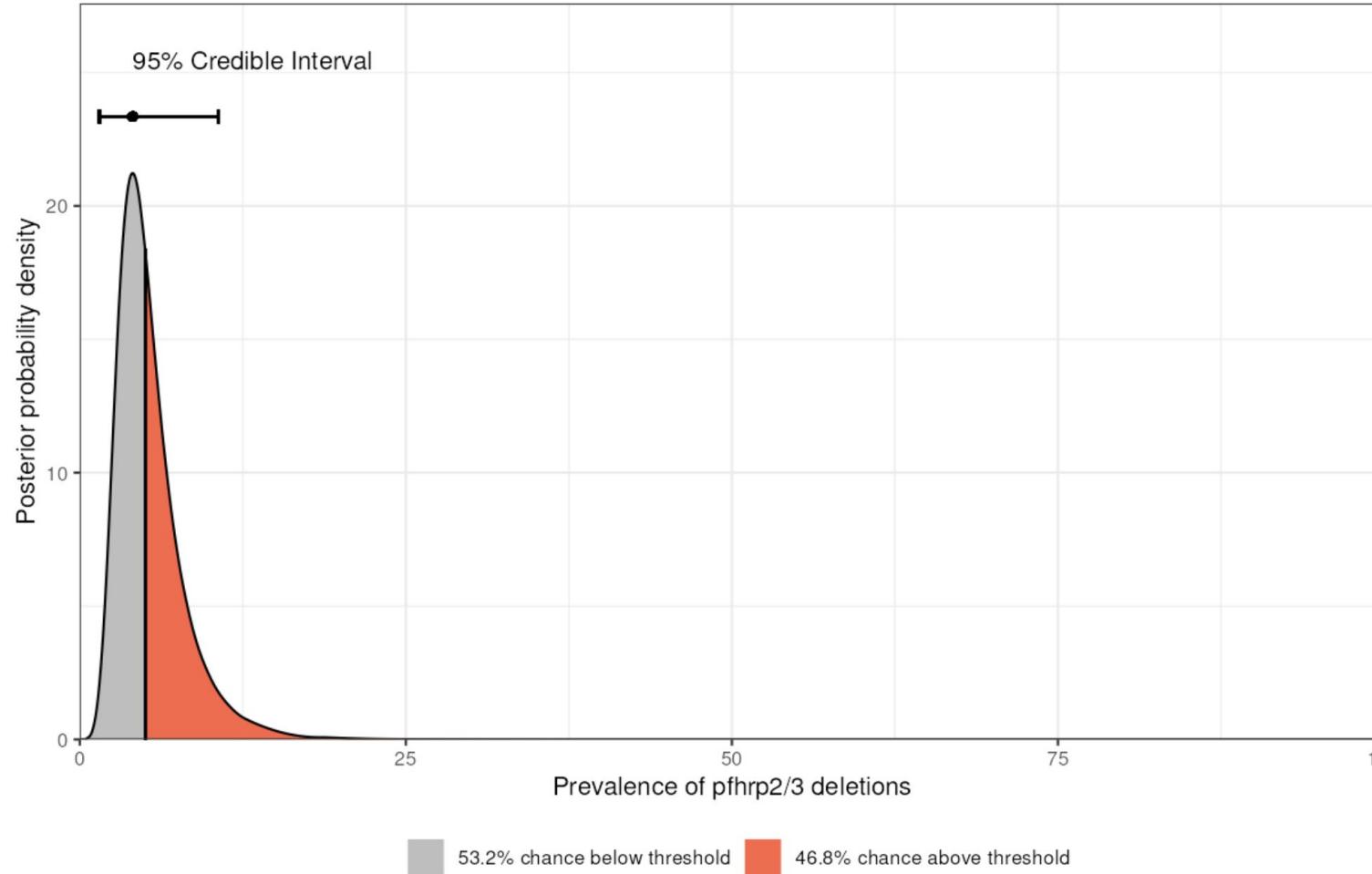
The table and the plot below show the maximum a posteriori (MAP) estimate of the prevalence, along with a 95% credible interval (Crl). The MAP estimate can be used as a central estimate of the prevalence, but it should always be reported alongside the Crl to give a measure of uncertainty.

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

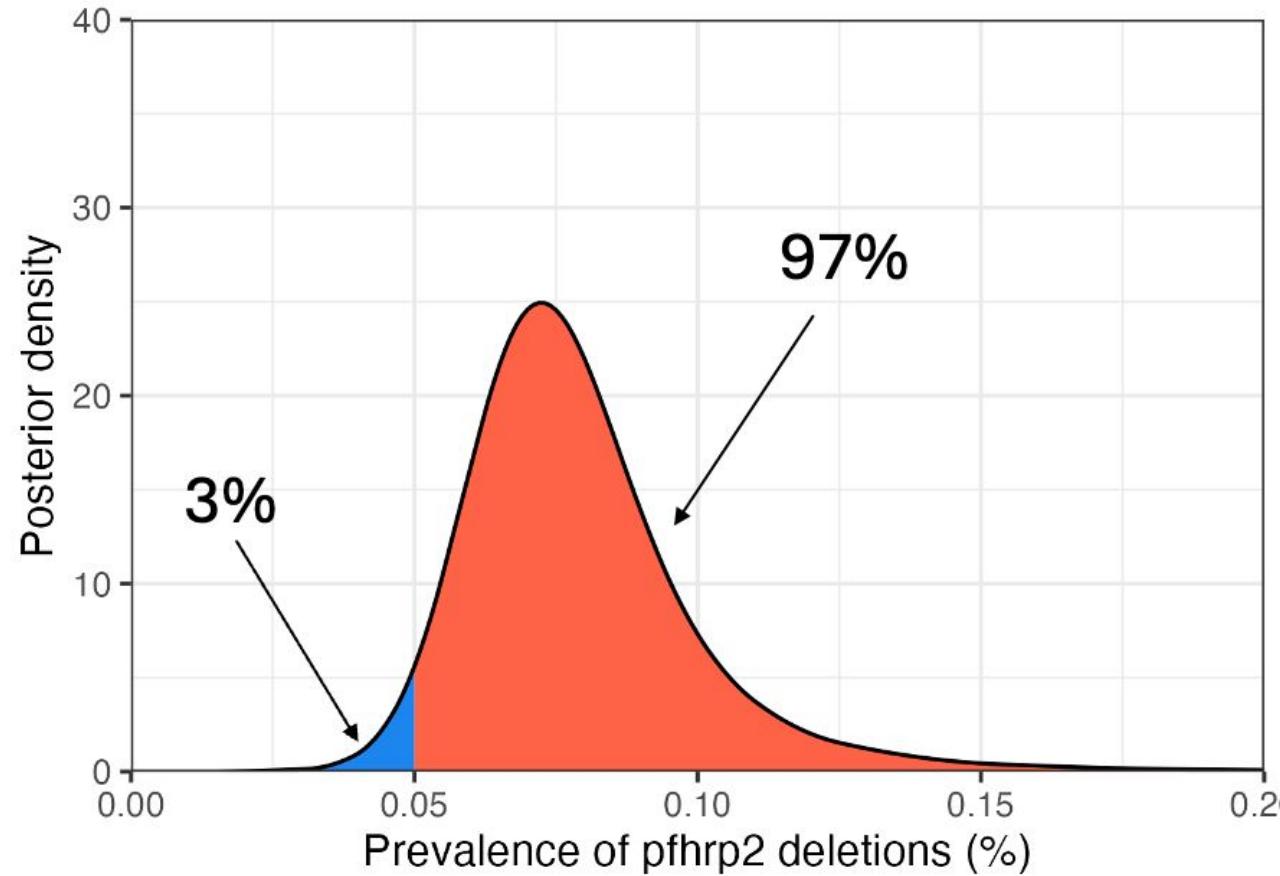
**RESULT:** We estimate that the prevalence of *pfhrp2/3* deletions is 4.05 % (95% Crl: 1.5 - 10.61 %). The probability that the prevalence is above the 5% threshold is estimated at 46.75 %. *We require greater than 95% probability to confidently conclude that prevalence is above the 5% threshold.*

**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

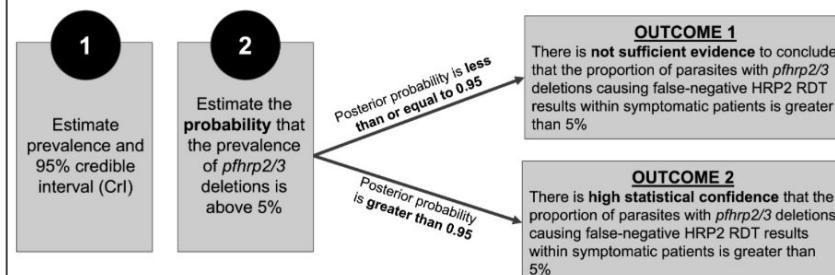
Downloaded on: 2024-07-02 | DRpower interactive app v1.0.1

### 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 and 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:

### STATISTICAL ANALYSIS FRAMEWORK



### 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:

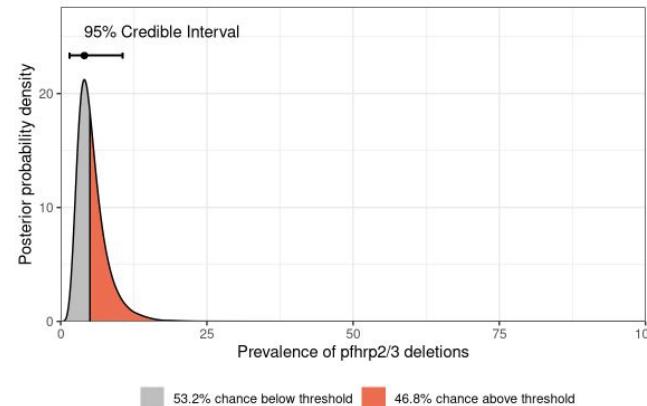
| Health facility              | Number of deletions | Sample size |
|------------------------------|---------------------|-------------|
| Northwestern Health Facility | 0                   | 60          |
| Northern Health Facility     | 0                   | 60          |
| Western Health Facility      | 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

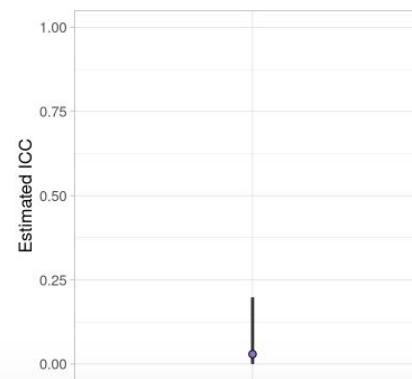
**RESULT:** We estimate that the prevalence of *pfhrp2/3* deletions causing false-negative HRP2 RDTs is 4.05% (95% CrI: 1.5-10.61%). The probability that the prevalence is above the 5% threshold is estimated at 46.75%. We require greater than 95% probability to confidently conclude that prevalence is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



- 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)

**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/](https://mrc-ide.github.io/MMS-SD_workshop/)