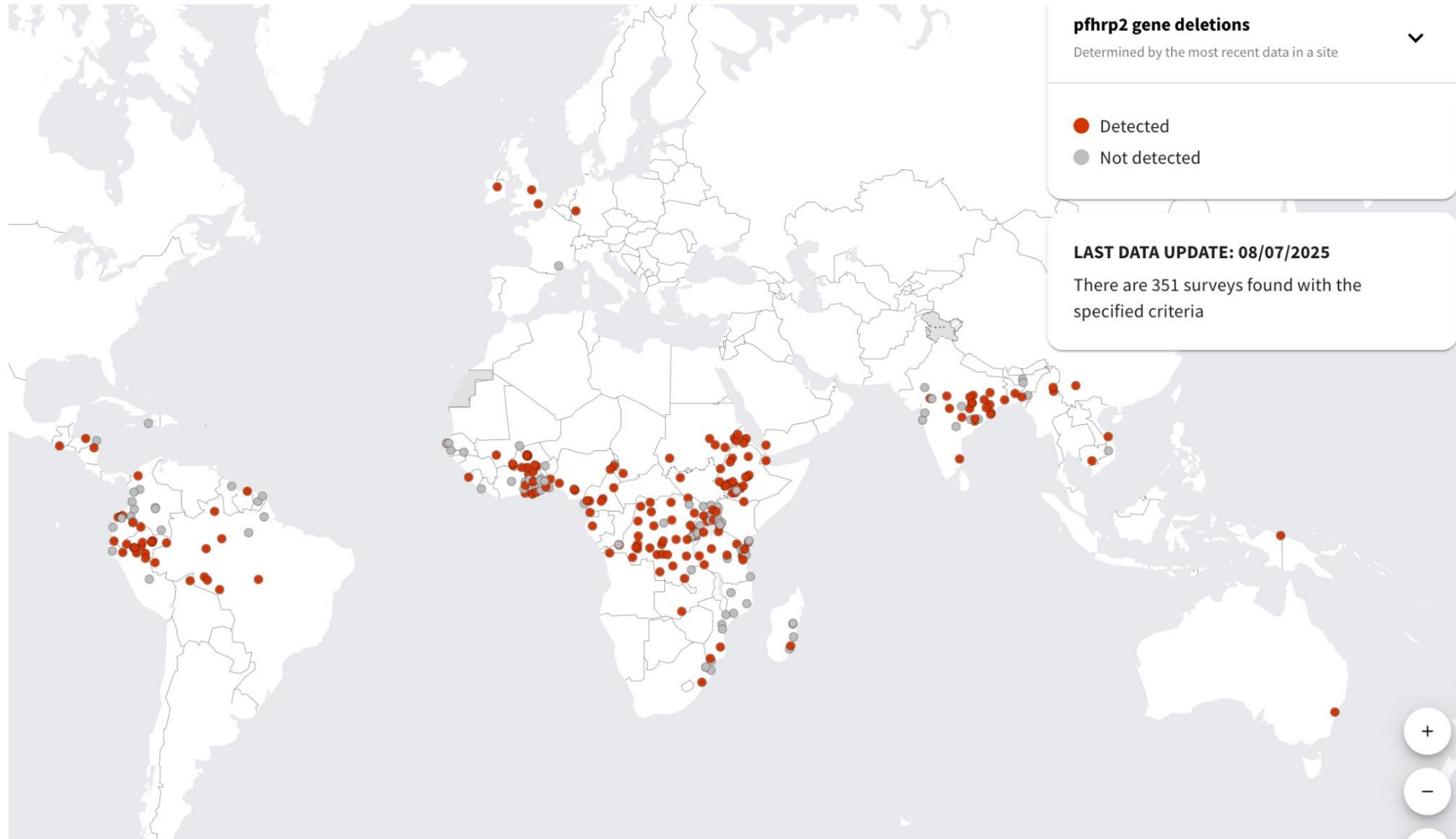


Malaria Molecular Surveillance Study Design Workshop

Module 6: The DRpower tool

Global landscape of *pfhrp2* deletions



The WHO master protocol



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 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

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

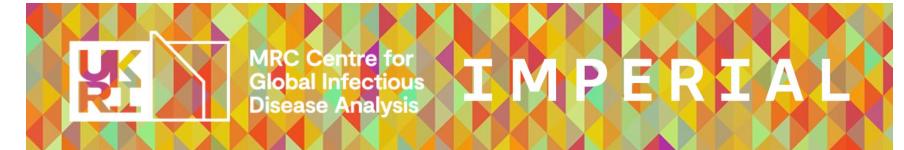
Each country will tailor protocol to their situation

- 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

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

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

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

Positive gold-standard test:

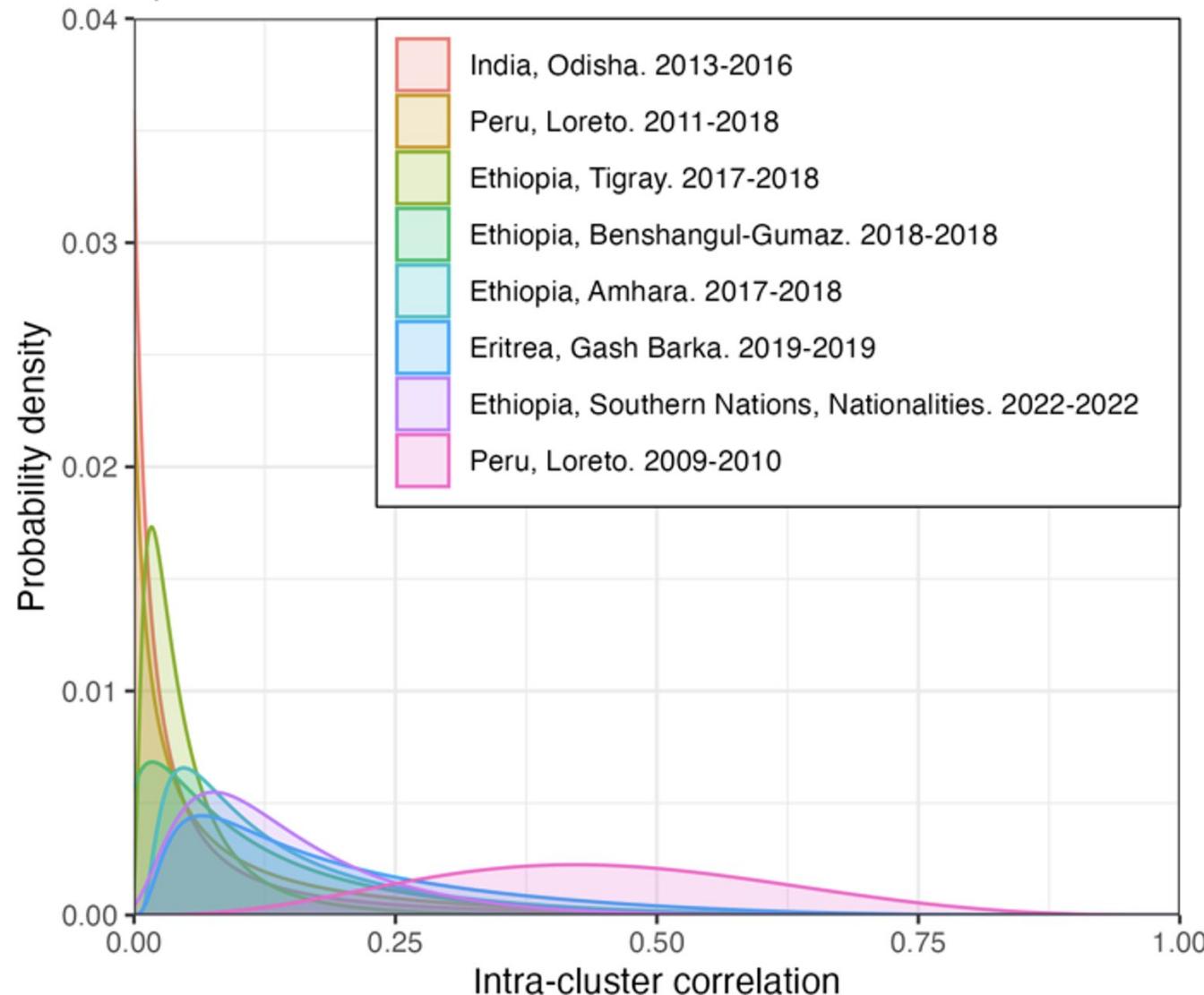
- Positive on the pan- or pf-pLDH test line, when a combination test is used
- Sample is confirmed microscopically to be positive for *P. falciparum* by two qualified microscopists

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%

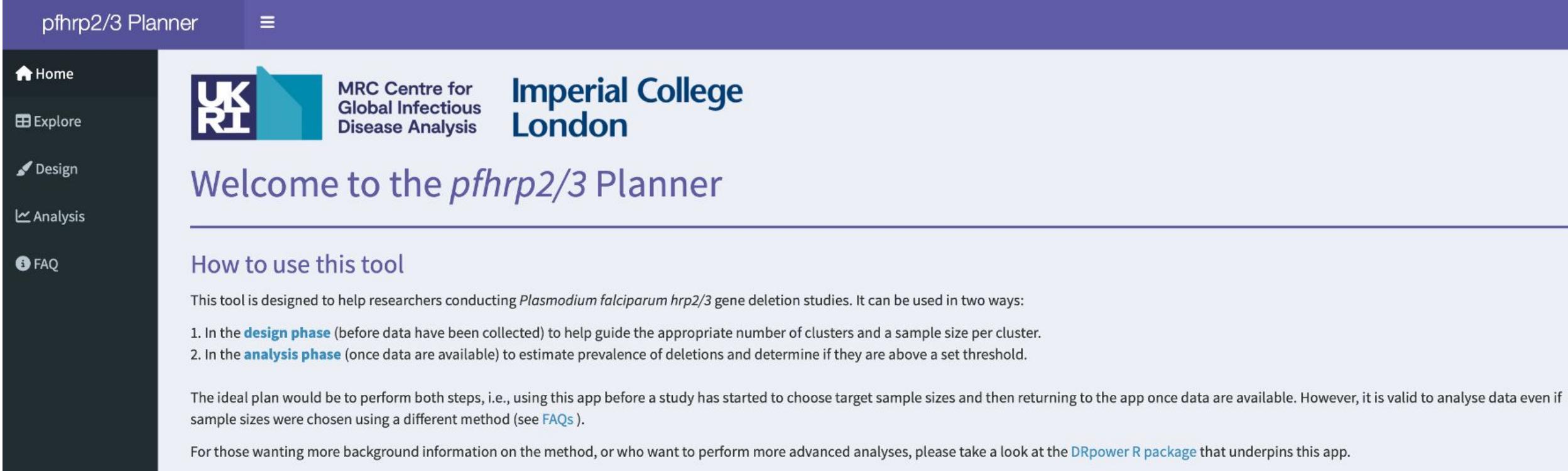
OUTCOME 2

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 grey header with the UKRI logo, 'MRC Centre for Global Infectious Disease Analysis', and 'Imperial College London'. Below this is a large blue header with the text 'Welcome to the pfhrp2/3 Planner'. The main body 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.

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.

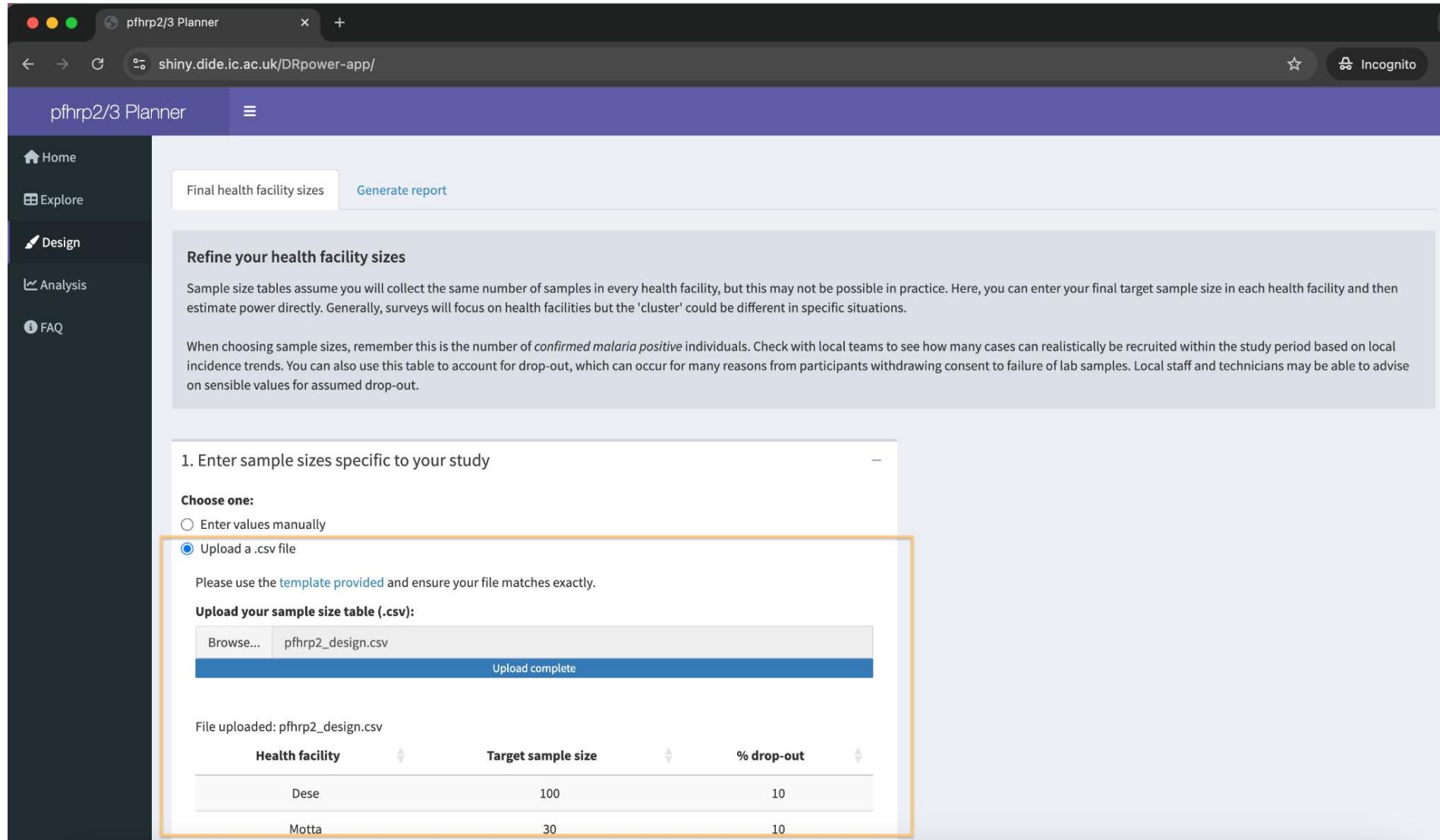
Explore sample sizes

- 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



The screenshot shows a web-based application titled "pfhp2/3 Planner" running in a browser. The URL in the address bar is shiny.dide.ic.ac.uk/DRpower-app/. The left sidebar has navigation links: Home, Explore, Design (which is selected), Analysis, and FAQ. The main content area has tabs: "Final health facility sizes" (selected) and "Generate report". Below these tabs is a section titled "Refine your health facility sizes". It contains two paragraphs of explanatory text. The first paragraph discusses sample size tables for health facilities, mentioning that surveys will focus on health facilities but the 'cluster' could be different in specific situations. The second paragraph advises users to consider confirmed malaria positive individuals and local incidence trends when choosing sample sizes, noting that local staff and technicians can advise on sensible values for assumed drop-out. The main form area is titled "1. Enter sample sizes specific to your study". It asks the user to "Choose one:" and provides two options: "Enter values manually" (radio button) and "Upload a .csv file" (radio button, which is selected). A note below the radio buttons says "Please use the template provided and ensure your file matches exactly." A "Browse..." button is followed by the file name "pfhp2_design.csv". A blue progress bar indicates the file is "Upload complete". Below this, a message says "File uploaded: pfhp2_design.csv". A table is displayed with three columns: "Health facility", "Target sample size", and "% drop-out". The data rows are "Dese" (Target sample size: 100, % drop-out: 10) and "Motta" (Target sample size: 30, % drop-out: 10).

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... pfhp2_design.csv Upload complete

File uploaded: pfhp2_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.

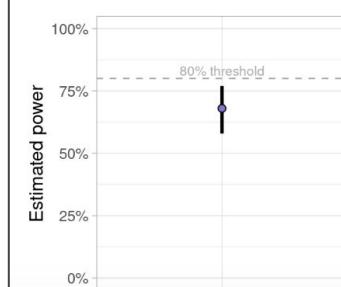
Health facility	Target sample size	Dropout (%)	Adjusted sample size
1	500	10	556
2	500	10	556
3	500	10	556
4	100	3	104
<i>Total samples</i>	<i>1600</i>	<i>–</i>	<i>1772</i>

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

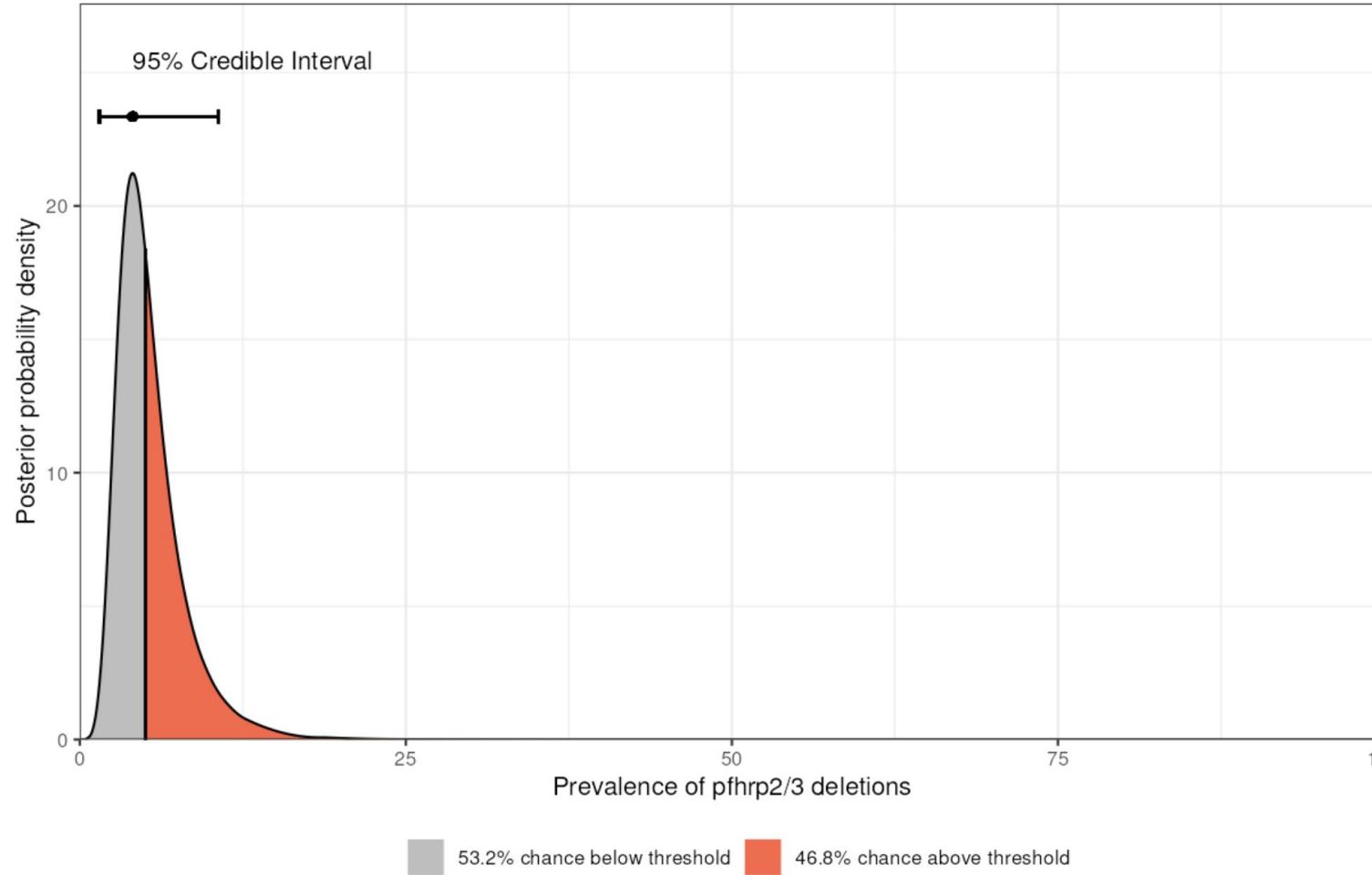
The table and the plot below show the maximum a posteriori (MAP) estimate of the prevalence, along with a 95% credible interval (Crl). The MAP estimate can be used as a central estimate of the prevalence, but it should always be reported alongside the Crl to give a measure of uncertainty.

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

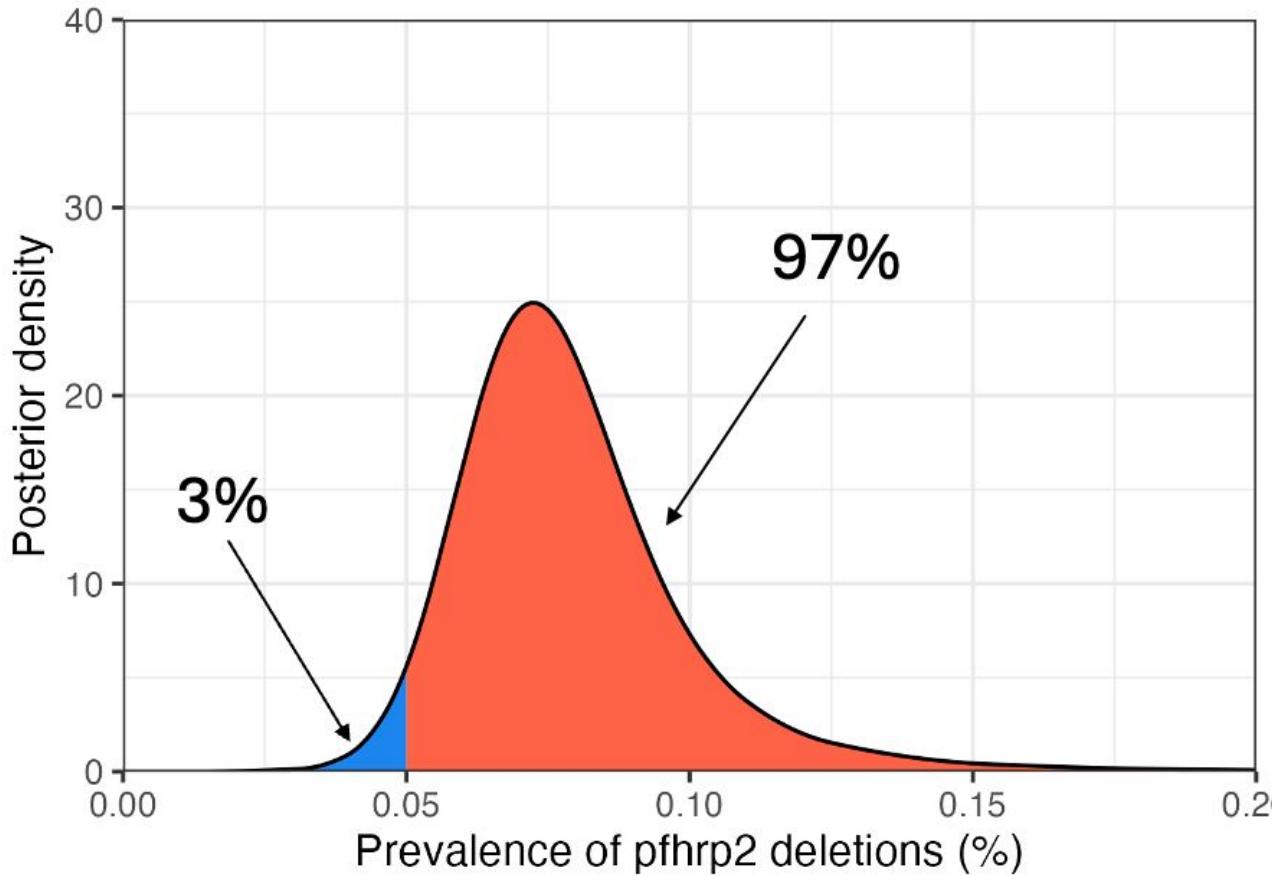
RESULT: We estimate that the prevalence of *pfhrp2/3* deletions is 4.05 % (95% Crl: 1.5 - 10.61 %). The probability that the prevalence is above the 5% threshold is estimated at 46.75 %. *We require greater than 95% probability to confidently conclude that prevalence is above the 5% threshold.*

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

pfhyp2/3 Planner: Analysis report

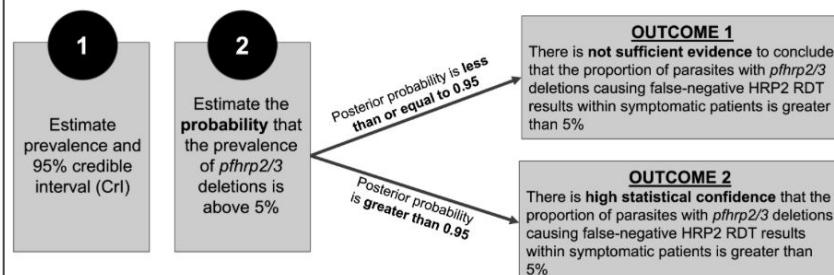
Downloaded on: 2024-07-02 | DRpower interactive app v1.0.1

Background

This report presents the results generated by the [pfhyp2/3 planner web application](#). This application was used to analyze data from a pfhyp2/3 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 pfhyp2/3 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 pfhyp2/3 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 pfhyp2/3 deletion prevalence study. The observed counts of pfhyp2/3 deletions causing false-negative HRP2 RDTs and the sample sizes in each health facility are shown below:

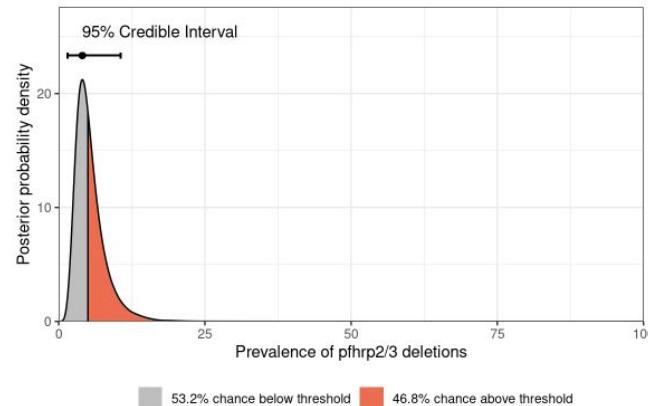
Health facility	Number of deletions	Sample size
Northwestern Health Facility	0	60
Northern Health Facility	0	60
Western Health Facility	1	60
Eastern Health Facility	2	60
Central Health Facility	5	60
Southern Health Facility	5	51
Southeastern Health Facility	3	51
Community Health Facility	0	20

Estimated prevalence

To estimate the prevalence of pfhyp2/3 deletions causing false-negative HRP2 RDTs, we used to test whether the observed prevalence in this study was above or below the

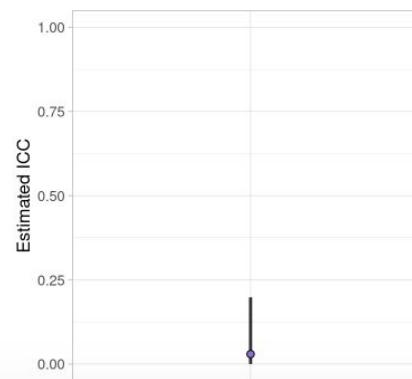
RESULT: We estimate that the prevalence of pfhyp2/3 deletions causing false-negative HRP2 RDTs is 4.05% (95% CrI: 1.5-10.61%). The probability that the prevalence is above the 5% threshold is estimated at 46.75%. We require greater than 95% probability to confidently conclude that prevalence is above the 5% threshold.

CONCLUSION: The prevalence of pfhyp2/3 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/