

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



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**World Health
Organization**

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Title	
Study site(s)	Site 1: Name, city, district and province Site 2: Name, city, district and province Site 3: Name, city, district and province <i>(Add more sites as needed or include just counties and add annex with health facilities list)</i>
Protocol submission date	dd/mm/yyyy
Protocol number	Unique protocol number/version number
Principal investigator	Name: Degree: Institution: Address: street, city, postal code, country Tel: Email:
Co-investigators (insert additional name(s) if needed)	Name: Degree: Institution: Address: street, city, postal code, country Tel: Email:
Participating institutions (insert additional institution(s) if needed)	Name: Complete postal address: street, city, postal code, country Tel: Email:
Planned study dates	From mmm/yyyy to mmm/yyyy
Sponsor	Ministry of Health, country Complete postal address: street, city, postal code, country Tel: Email:

Project summary

Objectives	<p>This survey is intended to determine whether the local prevalence of mutations in the <i>P. falciparum</i> <i>hrp2/3</i> genes causing false-negative RDTs has reached a threshold that might require a local or national change in diagnostic strategy. The specific objectives are to:</p> <ol style="list-style-type: none"> 1. Measure the prevalence of suspected false-negative HRP2 RDT results among symptomatic patients attending public health facilities with <i>P. falciparum</i> infection detected by microscopy or a pf-pLDH RDT; 2. Detect the parasite density and frequency of <i>pfhrp2/3</i> gene deletions in that cohort; 3. Determine the predictive value of false-negative HRP2 RDT results for <i>pfhrp2/3</i> gene deletions in different settings; 4. Identify provinces in which the prevalence of <i>pfhrp2/3</i> gene deletions causing false-negative <i>P. falciparum</i> RDTs is at or above 5%, warranting a change in RDTs. <p>The biobanking activity is intended to support future malaria epidemiological research and the development of new and/or improved health products particularly those targeting <i>pfhrp2/3</i> deleted parasites.</p>
Surveillance site	Pre-selected public health facilities representing the spectrum of transmission and geographical diversity across the country
Target population	Individuals meeting case definition for suspected malaria case
Survey type	Cross-sectional, multi-site
Primary output measures	<ol style="list-style-type: none"> 1. Prevalence of suspected false-negative HRP2 RDT results among symptomatic patients with <i>P. falciparum</i> malaria 2. Prevalence of <i>pfhrp2/3</i> gene deletions among symptomatic falciparum patients with a false-negative HRP2 RDT result 3. Prevalence of <i>pfhrp2/3</i> gene deletions causing false-negative HRP2 RDTs amongst all symptomatic <i>P. falciparum</i> confirmed cases
Secondary output measures (optional)	<ol style="list-style-type: none"> 1. Parasite density, as measured by quantitative PCR and/or microscopy, in patients with suspected false-negative HRP2 RDT results. 2. New knowledge concerning the epidemiology of <i>pfhrp2/3</i> deletions eg. genetic diversity and drug resistance status and new and improved tools to diagnose <i>pfhrp2/3</i> deleted parasites.
Sample size	A sample size of 600 confirmed <i>P. falciparum</i> cases per sampling domain (60 per health facility) is recommended to quantify whether or not the prevalence of <i>pfhrp2</i> deletion is above 5%. Once the sample of 600 <i>P. falciparum</i> cases have been enrolled then molecular confirmation of <i>pfhrp2</i> deletions amongst suspected false-negative <i>P. falciparum</i> cases should ensue.

Sampling method	In at least 10 pre-selected health facilities per sampling domain eg. province at risk, a cross-sectional survey will measure the suspected and confirmed prevalence of <i>pfhrp2/3</i> gene deletions causing false-negative HRP2 RDT results. 60 <i>P. falciparum</i> confirmed cases should be included in each health facility.
Data collection	<ol style="list-style-type: none"> 1. Identify provinces to be included in the study. 2. Select at least 10 health facilities per province for testing (facility sample size may vary depending on logistical and budgetary constraints). Any facility where RDTs are being used is eligible; however, microscopy services are not a requirement. 3. In the target population (suspected malaria cases), conduct routine case management procedures and obtain informed consent (or assent depending on the age of majority and federal guidelines in the country of enrolment), to perform an additional RDT and collect a dried blood spot (DBS) for laboratory analysis and for biobanking/long term storage to support future malaria epidemiological and diagnostic research. 4. Take clinical history including questions regarding age, sex, recent malaria diagnostic testing, antimalarial therapy and travel. 5. Test all consenting suspected malaria cases simultaneously using both a WHO-recommended HRP2 RDT and a non-HRP2 method (e.g., pf-pLDH RDT (separate single or multiple test line RDT) or quality –assured microscopy in the health facility and collect minimum two blood spots on filter paper or protein saver cards. 6. Record demographic and clinical history details and all test results on the survey report form. 7. Administer antimalarial therapy based on results from (either) RDT and/or microscopy and according to national guidelines. 8. Retain used RDTs for quality control and minimum two DBS from all consenting Pf patients for molecular +/- serological analysis. 9. Enrolment can stop once 600 individuals with confirmed <i>P. falciparum</i> malaria (ideally ~37/site across the 10 sites in the province), have been recorded in the survey tally sheet as having <i>P. falciparum</i>. 10. Ship all consent forms, tally sheets, survey report forms and patient samples to the central coordinating centre. 11. Central laboratory staff review survey report forms and identify the suspected <i>pfhrp2/3</i> deletion cases and prioritize these DBS for molecular +/- serological analysis. 12. Proceed with supplemental data analysis according to a prioritization and resources available ie. HRP2 positive samples, and HRP2 and pf-LDH negative samples, as options are described in Appendix 1.

13. Discard all RDTs, microscopy slides and DBS after survey results finalized and reported, unless consent for long term storage of DBS is obtained.

Statistical and analytic plan

The prevalence of suspected false-negative HRP2 RDT results and *pfhrp2/3* gene deletions will be established at the sampling domain (e.g. provincial level), with 95% confidence intervals (CI) estimated for all point estimates. If desired, point estimates and 95% CIs can be weighted according to relative facility size or patient flows. Differences between point estimates across sociodemographic characteristics and transmission levels, or other collected variables can be determined using χ^2 and/or logistic regressions, as desired.

1. Background and rationale

Rapid diagnostic test (RDT) kits offer great potential for the immediate diagnosis of malaria infections. Rapid diagnosis allows for prompt treatment, especially in rural settings. RDTs are lateral flow immunochromatographic tests that detect *Plasmodium* parasite antigens in blood [1]. Three antigens are detected by current RDTs: histidine rich protein 2 (HRP2), lactate dehydrogenase (LDH) and aldolase. HRP2 is an abundant protein expressed only by *P. falciparum* and is the target for the most commonly used RDTs. Although the antibodies on the test strip are designed to recognize the HRP2 antigen, they may also cross-react with another antigen of the HRP family, namely HRP3, due to strong similarities in the amino acid sequences [2]. HRP2-based RDTs tend to be more sensitive and heat-stable than RDTs that detect LDH or aldolase [3].

While HRP2 RDTs generally have the highest sensitivity of the RDTs for *P. falciparum* malaria [3], parasite strains have recently been identified that have deletions in the genes encoding HRP2 or the similar HRP3 protein. Strains with *pfhrp2* and *pfhrp3* gene deletions are undetectable by HRP2 RDTs [4]. HRP2 RDTs can sometimes still detect strains with only a *pfhrp2* deletion, particularly in high parasite density infections, due to antibody cross-reactivity with epitopes of HRP3 [4]. In 2010, Gamboa et al. [5] first reported the identification of *P. falciparum* parasites with *pfhrp2/3* gene deletions in the Amazon River basin in Peru. Subsequent retrospective analyses at different sites in the Loreto region of the Peruvian Amazon showed an increase in the prevalence of parasites with gene deletions between specimens collected from 1998 to 2001 (20.7%) and those collected from 2003 to 2005 (40.6%) [5]. The prevalence of parasites with *pfhrp2/3* gene deletions shows substantial local variability. Studies in other countries, such as India [6], Mali [7], Honduras [8], Ghana [9], Columbia [10, 11] Myanmar [12], Suriname [13], Guyana [8] and Senegal [14], have found much lower prevalence estimates, although the rigour of study design has been variable. In recently published data from Eritrea, the prevalence of dual *pfhrp2* and *pfhrp3* deletions was found to be very high (80%), requiring an urgent response and policy change away from HRP2-only testing strategy [15]. There have been no reports of parasites failing to express LDH or aldolase, as these targets are essential enzymes for parasite metabolism and survival.

In settings where microscopy is either unavailable or infeasible due to time or resource constraints, it is imperative that malaria be treated based on RDT results. Monitoring the accuracy of the RDT results is thus critical. The main causes of false-negative RDT results are related to product quality and performance, transportation or storage conditions, operator error, or parasite density below the limit of detection; however, deletions of the genes encoding the target antigen must also be considered [4]. To avert a crisis like the one that emerged in Eritrea in 2016, WHO recommends that countries with any reports of *pfhrp2/3* deletions as well as neighbouring countries conduct surveillance for *pfhrp2/3* deletion particularly amongst symptomatic patients [3].

The purpose of this document is to present a standardized protocol that *P. falciparum*-endemic countries can use to identify the prevalence of parasites with *pfhrp2/3* gene deletions causing false-negative HRP2 RDT results among symptomatic falciparum patients. The methods contained herein can be used to map the distribution of these deletions, estimate the predictive value of suspected false-negative HRP2 RDT results for gene deletion, and identify areas where diagnostic strategies may need to be changed.

2. Survey and research objectives

This survey is primarily intended to determine whether the local prevalence of deletions in the *P. falciparum* *hrp2/3* genes causing false-negative HRP2 RDT results among symptomatic falciparum

patients has reached a threshold that might require a national or subnational change in malaria RDTs. The specific objectives are to:

1. Measure the prevalence of suspected *pfhrp2/pfhrp3* gene deletions among symptomatic falciparum patients attending public health facilities.
2. Detect the parasite density and frequency of *pfhrp2/3* gene deletions in that cohort.
3. Determine the predictive value of suspected false-negative HRP2 RDT results for *pfhrp2/3* gene deletions in different settings.
4. Identify provinces that have 5% or higher prevalence of *pfhrp2/3* gene deletions in that cohort, as this indicates a need to switch from using exclusively HRP2-based RDTs for detecting *P. falciparum*.
5. Support future malaria epidemiological research and product research and development for malaria.

3. Survey site(s) / target population

This surveillance activity will focus on suspected malaria cases seeking care at public health facilities. Negative HRP2 RDT results but pf-positive results by pf-pLDH RDT or microscopy indicate the possibility of *pfhrp2/3* gene deletion as the reason for the false-negative result. Given the importance of HRP2 detection to the diagnostic strategy, WHO is urging at-risk countries to assess the prevalence of such *P. falciparum* gene deletions. Prioritized for surveillance are areas (i) with a recognized discordance between HRP2 RDT and microscopy results, (ii) with non-representative or sporadic reports of *pfhrp2/3* deletions in the country, and (iii) that neighbour an area where frequent *pfhrp2/3* deletions have been identified. In such countries, public health facilities within all provinces with *P. falciparum* malaria transmission should be included. Facilities eligible for inclusion in the study should ideally represent the geographical spread of malaria transmission across the province.

3.1 Inclusion criteria

- Meet case definition for suspected malaria case

3.2 Exclusion criteria

- Previously enrolled in the survey

4. Survey methods

4.1 Design

A cross-sectional survey design will be used to measure the primary outputs. Health facilities will systematically test suspected malaria cases with an HRP2 RDT and an alternative method (i.e., pf-pLDH RDT or microscopy) and collect a minimum two DBS. The frequency of suspected false-negative HRP2 RDT results among symptomatic patients with *P. falciparum* malaria is primary output 1. Molecular testing on the DBSs from suspected false-negative HRP2 RDT results will determine the prevalence of *pfhrp2/3* gene deletions in HRP2 RDT false-negative cohort (output 2) and in the cohort of all symptomatic *P. falciparum* confirmed cases (output 3).

4.2 Primary output indicators

The following indicators will serve as the primary survey outputs:

1. Prevalence of suspected false-negative HRP2 RDT results (i.e., a negative HRP2 RDT result but positive pf-pLDH or Pf microscopy result and Pf PCR positive) 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 survey will identify the proportion of patients with suspected false-negative HRP2 RDT results through diagnostic testing at health facilities using dual-method testing (HRP2 RDT plus microscopy or pf-LDH RDT¹). To save time and resources, molecular +/-serological testing to confirm *P. falciparum* infection and identify *pfhrp2/3* deletions will only initially be performed on DBSs collected from individuals with these suspected false-negative HRP2 RDT results. Discordant diagnostic results may be due to other factors, such as false-positive pf-pLDH test lines (possibly due to cross-reactivity with non-falciparum species) or low parasite densities at or below the limit of detection of the HRP2 and pf-pLDH RDTs. In addition, there are several situations in which this indicator could miss a true *pfhrp2/3* gene deletion (see Table 3 below). First, individuals will not be detected if they have a low-density infection that is missed by pf-pLDH RDT or microscopy and also by HRP2 RDT due to *pfhrp2/3* gene deletion. Second, HRP2 RDT may still detect some infections with a *pfhrp2* deletion due to cross-reactivity of test antibodies with HRP3. Finally, the testing protocol will not detect *pfhrp2/3* deletions in patients coinfecting with HRP2-expressing clones unless novel techniques are used such as deep sequencing and digital PCR. For these reasons, this indicator represents the lower limit of the true prevalence of *pfhrp2/3* gene deletions.

4.3 Secondary output indicators (optional)

1. Parasite density, as measured by quantitative PCR and/or microscopy, in patients with suspected false-negative HRP2 RDT results.
2. Improved understanding of *pfhrp2/3* gene deletions eg. genetic relatedness and/or drug resistance, fitness and new and/or improved diagnostic tools for detecting *pfhrp2/3* deleted parasites.

4.4 Sample size

Sample size is based on the desire to obtain relatively precise estimates of false-negative HRP2 RDT results caused by *pfhrp2/3* gene deletions at the survey domain level (province, state or similar) within countries implementing this protocol. The sample size estimates are based on a proportion obtained from simple random sampling, with a sampling design effect (deft) = 1.5 (to account for observations correlated within clinics vis-à-vis *pfhrp2/3* gene deletions) and a probability of committing a type-1 error = 95% (1-sided test), such that the 95% confidence interval does not overlap with the threshold of 5%. Note that a design effect other than 1.5 can be used if data from clustering of *pfhrp2/3* (or other relevant output) exist that can be used to estimate a design effect.

¹ RDTs should be used that contain pf-pLDH-specific test lines and not pan-pLDH test lines. This will ensure that only *P. falciparum* infections are detected and avoid the identification of non-falciparum species (Pv, Pm, Po) species, which would cause discordant results (HRP2 negative, pan-pLDH positive)

$$n \geq \text{def} \left[\frac{Z^2(P)(1-P)}{D^2} \right]$$

Sample size is based on estimating output indicators 1 and 2,² where the upper bound of the 95% CI does not overlap with 5% for estimates in which the observed prevalence of false-negative HRP2 RDT results caused by *pfhrp2/3* gene deletions is below 5% (signaling that the observed level of *pfhrp2/3* gene deletion is below 5% with 95% confidence), and where the lower bound of the 95% CI does not overlap with 5% for estimates in which the observed prevalence is above 5% (signaling that the observed level of *pfhrp2/3* gene deletion is above 5% with 95% confidence).

During the statistical analysis, health facilities will be included as a random effect so that the prevalence estimates and the 95% CI are adjusted for the variability in the probability of finding a malaria case at a health facility.

To demonstrate that the prevalence of *pfhrp2/3* gene deletion (causing false-negative RDTs) within symptomatic patients with *P. falciparum* is below or above 5%, a sample size based on an expected population prevalence of 3.2% (n=584, rounded up to 600) or 8.0% (n=500), respectively, per sampling domain would be adequate. As a minimum, therefore, a sample of 600 individuals with a *P. falciparum* infection per sampling domain (e.g. province) is recommended (60 per health facility, 10 health facilities per sampling domain) based on the formula above. Within the domain, health facilities should be selected on the basis of probability proportional to size depending on the fever or suspected malaria caseload.

As the prevalence of *pfhrp2* deletions gets closer to the 5% threshold, detecting if it is above or below the threshold requires an increasingly large sample size, up to a maximum sample size exceeding 30,000 *P. falciparum* malaria cases per domain when an estimate within 5% +/- 0.2 % is made, based on the formula above.

Therefore, it is recommended to conduct the survey first with a sample of 600 per domain. Molecular analysis should then be undertaken on the DBS samples suspected to have *pfhrp2/3* deletions and a statistical analysis of the prevalence with 95% CI computed. The analysis will result in one of three outcomes per province:

Outcome 1: The estimated proportion is lower than 5% and the upper limit of the 95% CI is below 5%. In this case there is a high statistical confidence that the proportion of parasites with *pfhrp2/3* deletions causing false-negative HRP2 RDT results within symptomatic Pf patients is below 5%

Outcome 2: The estimated proportion is higher than 5% and the lower limit of the 95% CI is above 5%. This result means that there is a high statistical confidence that the proportion of *pfhrp2/3* deleted parasites causing false-negative RDT results in symptomatic Pf patients is greater than 5%

Outcome 3: The statistical analysis shows that it is inconclusive (5% contained within the 95% CI) as to whether or not the prevalence of *pfhrp2/3* deletion causing false-negative RDT results in symptomatic Pf patients is greater than or less than 5%.

4.5 Sampling

In general, in each province, a systematic random sample of a minimum of 10 health facilities should be selected from a complete list of all facilities, stratified by facility type and including a measure of

² Proportion of all *P. falciparum* malaria patients who have suspected false-negative HRP2 RDT results (positive on pf-pLDH RDT or microscopy and negative on HRP2 tests); proportion of all *P. falciparum* malaria patients with suspected false-negative HRP2 RDT results found to have *pfhrp2/3* gene deletions.

facility size (e.g., number of fevers or suspected malaria outpatients seen at the health facility in an average month). For each province, the selection of facilities should be based on the relative size of each facility, so that sampling is based on probability proportional to size (PPS). Budget permitting, more than 10 health facilities per province can be used to recruit the required sample in the very low endemic counties; this will increase estimate precision and decrease the length of time needed to meet enrolment targets.

Create a map of the sampling domains selected across epidemiological zones and the proportion of health facilities within each one.

Create a table that provides a list of all enrolling health facilities per sampling domain

4.6 Data collection and fieldwork

The following general steps for data collection will be followed and outlined in a figure ***[Note, it will be necessary for each country to develop a specific standard operating procedure (SOP) in order to tailor these steps to their particular context and needs].***

The National Malaria Control Programme (NMCP) should identify provinces to be surveyed. This selection should be guided by an assessment of which provinces may be most at risk for *pfhrp2/3* gene deletions otherwise a random selection of counties has been selected based on epidemiological zones. Provinces with low, moderate and high transmission should be considered, whereas provinces without malaria transmission should be omitted. The sample sizes may be reached more quickly in moderate to high transmission areas; however, the expected higher prevalence of multi-clone infections may mask the presence of *pfhrp2/3* deleted parasites. This would be less likely in low transmission areas and therefore these zones should be included

1. Select a specified number of public health facilities (minimum of 10) in each province of the country routinely using malaria RDTs, to be included in the survey.
 - The number of facilities per province to be included in the sample should take into account the expected mean number of suspected malaria patients seen in the facility each week and the mean test positivity rate in the target area in order to ascertain the expected number of positives each week. As a general rule, the aim is to finish the fieldwork and collect a minimum sample size of 600 positives within an 8-week period.
 - Health facilities for the sample should be selected from a complete list (sampling frame) of health facilities in each province, using systematic random sampling based on PPS [17] (and proportional to the size of the facility type strata in each domain). The sampling frame must include some estimate of facility size (fever or suspected malaria case load) and type (e.g., public, private, level, etc.).
 - *Note that if budgetary or logistical constraints preclude the selection and inclusion of facilities using random sampling, a purposeful (or convenience) sample of facilities can be used. However, it should then be noted that province-level estimates of pfhrp2/3 gene deletions will not be statistically representative of the province.*
2. Survey procedures and analysis of data
 - a. Patients are triaged according to normal procedures. Any patient considered by the routine health provider eg. physician, nurse, to be a suspected malaria case, according to national guidelines, will be asked for consent/assent (Appendix 3,4).

- b. Only consenting individuals will sign their name and be given a copy of the information sheet with their survey/sample ID in case they change their mind and wish not to participate in the future or have samples removed from storage;
 - c. Consenting patients will be asked a series of questions relevant to their illness and tested simultaneously according to manufacturers instructions for use by two separate RDTs, including the HRP2 RDT used in the national control programme and a pf-pLDH, as well a minimum of two blood spots will be collected on filter paper/protein saver cards.
 - d. Non-consenting patients will receive routine care, no record of their name or identity will be appear on any survey documents. The blank non-consent form will be assigned a unique ID, to allow for monitoring of the total number consenting and non-consenting suspected malaria cases attending the facility on the survey tally sheet (Appendix 2). All informed consent forms will be kept in a secure location under lock and key.
 - e. The HRP2 RDT and the pf-LDH RDTs meet WHO procurement criteria and have been approved for use by the Ministry of Health.
- A pf-pLDH RDT that meets WHO procurement criteria (Table 1) or microscopy can be used for the secondary diagnosis. At present, there are no RDTs that meet the WHO performance criteria for detection of *P. falciparum* based on their pf-LDH test line alone. [16]; therefore, for the purposes of the survey, countries may exceptionally select pf-pLDH-based RDTs that (i) have been evaluated in WHO malaria RDT product testing; ii) meet performance criteria for detection of HRP2 expressing *P. falciparum* and (iii) have a PDS >90 at 2000p/μl, and false-positive and invalid rates <2% (see Table 1).
 - If microscopy is to be used, prepare one thin and one thick blood film following national guidelines aligned with the WHO SOPs for malaria microscopy.³

³http://www.wpro.who.int/mvp/lab_quality/mm_sop/en/

TABLE 1.

WHO recommended malaria RDT options for detection of both HRP2-expressing and non-expressing *P. falciparum* malaria for case for *pfhrp2/3* gene deletion surveillance

Performance criteria**(highlighted in green if met):**A: *P. falciparum* panel detection score (PDS)^a ≥ 75% at 200 parasites/μLB: *P. vivax* panel detection score (PDS)^a ≥ 75% at 200 parasites/μL

C: false-positive (FP) rate against clean negatives < 10%

D: invalid rate (IR) < 5%

E: *pfhrp2* negative *P. falciparum* panel detection score (PDS) > 75% at 200 parasites/uL (in areas where *pfhrp2* deletions are prevalent)

			<i>P.falciparum</i> HRP2 expressing, <i>P.vivax</i> and malaria negative panels				<i>P.falciparum</i> HRP2 non-expressing panel				
Product	Manufacturer	WHO Prequalification status	PDS ^a		FP	IR	PDS	Pf @2000p/μL ^h	Meets WHO performance criteria for detection of <i>pfhrp2/3</i> deleted <i>P.falciparum</i> ⁱ	Applicable for use with a HRP2 RDT, as screening tool for surveys of <i>pfhrp2</i> deletions, if there are no better alternatives ^j	
			A ^b	B ^c	C ^d	D ^e	E ^g				
Pf only											
BIOCREDIT Malaria Ag Pf (pLDH)	C14RHA25 C14RHG25 C14RHH25	RapiGEN Inc	In pipeline, passed lab evaluation	*	*	*	*	*	Yes	Yes	
BIOCREDIT Malaria Ag Pf (pLDH/HRPII)	C13RHA25 C13RHG25 C13RHH25	RapiGEN Inc	In pipeline, passed lab evaluation	*	*	*	*	*	Yes	Yes	
SD BIOLINE Malaria Ag P.f (HRP2/pLDH) ^f	05FK90	Abbott Diagnostics Korea Inc	Prequalified	90 (88/71) ^g	NA	0.0	0.1	57.5 (0/57.5) ^h	100	No	Yes
Pf and Pv											
BIOCREDIT Malaria Ag Pf/Pv (pLDH/pLDH)	C61RHA25 C61RHG25 C61RHH25	RapiGEN Inc.	In pipeline, passed lab evaluation	*	*	*	*	*	Yes	Yes	
Pf, Pf and Pv											
SD BIOLINE Malaria Ag P.f/P.i/P.v ⁱ	05FK120	Abbott Diagnostics Korea Inc	Prequalified	89 (89/62) ^g	97.1	0.0	0.0	42.5 (0/42.5) ^h	100	No	Yes

* specific results unavailable until product is WHO prequalified; UK – unknown; Pf, *Plasmodium falciparum*; Pv, *Plasmodium vivax*

a - A sample is considered detected only if all RDTs from both lots read by the first technician, at minimum specified reading time, are positive

b - Round 1, n=79; Round 2, n=100; Round 3, n=99; Round 4, n=98; Round 5, n=100; Round 6, n=100; Round 7, n=100; Round 8, n=100

c - Round 1, n=20; Round 2, n=40; Round 3, n=35; Round 4, n=34; Round 5, n=35; Round 6, n=35; Round 7, n=35; Round 8, n=35

d - Round 1, n=168; Round 2, n=200; Round 3, n=200; Round 4, n=232; Round 5, n=236; Round 6, n=208; Round 7, n=220; Round 8, n=208

e - Round 1, n=954; Round 2, n=1240; Round 3, n=1204; Round 4, n=1192; Round 5, n=1214; Round 6, n=1210; Round 7, n=1210; Round 8, n=1210

f- PDS presented in the table is based on a positive Pf test line (either HRP2 or Pf-LDH). The results in brackets are the PDS based alone on HRP2 and Pf-LDH test lines, respectively.

g Round 8, n=40 (18 double deletion: *pfhrp2*-/*pfhrp3* -; 22 single deletion; *pfhrp2*-/*pfhrp3*+)

h - Results (PDS) of adhoc assessment of pLDH containing round 8 RDTs against high density HRP2 negative panel: n=40 (18 double deletion: *pfhrp2*-/*pfhrp3* -; 22 single deletion; *pfhrp2*-/*pfhrp3*+)

i These results should be considered when procuring RDT for use in areas where *pfhrp2* + or - *pfhrp3* deletions are prevalent.

j - RDTs including pf-LDH individual test lines that have a PDS >90% against *pfhrp2* deleted parasite samples of 2000 parasites/μL may be used to screen for *pfhrp2* deletions as per this WHO survey protocol template

For each consenting suspected malaria case the health provider will attach a unique survey ID, chronologically ordered, to a survey case report form (Appendix 5), and to the survey tally sheet. On the survey report form the health worker will record answers to questions including age, sex, recent history of malaria diagnostic testing, treatment and travel.

- Next, using the labels attached to the survey case report form (and sharing the same ID number as the form) the health worker (treating clinician or laboratory worker) will label 2 different RDTs or 1 RDT and microscopy slide and a filter paper or protein saver card for DBS. He/she will perform the tests, record results on the survey case report form (Appendix 5, sections 5-7) and inform patient of results directly (clinician) or refer the patient back to the treating clinician (laboratory worker), to provide treatment for positive test results on the primary or secondary RDT, or microscopy as per national guidelines. Results from either RDT should be acted upon as both are WHO prequalified, furthermore, microscopy is also considered an acceptable alternative to malaria RDTs. Negative RDT results should be managed as per national guidelines.
3. All used RDTs and, if applicable, microscopy slides from each consenting suspected malaria case will be stored until the survey is completed in a dry and protected area for survey quality control purposes.
 4. The minimum of two DBSs (50µl per spot) on filter paper or protein saver card should be placed in a clean, dry and protected area, and allow to dry for 3-4 hours.
 - Once dry, the filter paper/cards will be placed with the desiccant (from the RDT package) in an impermeable plastic bag, labelled with survey ID if it is not stuck directly)
 5. Once the desired sample size of infected, consenting individuals is obtained at each facility the survey case report forms and corresponding, RDTs and DBS should be compiled and sent to the central coordinating centre. No names or other unique identifying information is contained on forms, tally sheets, RDTs or DBSs. The only link between patient name and survey ID number is on the consent form of consenting individuals.
 6. Upon receipt of forms/RDTs/DBSs, the survey team supervisor must review the report form RDT results section (Appendix 5, section 5-7, S1) determine which DBSs to prioritize for molecular +/- serological analysis, specifically, the HRP2 RDT negative and pf-LDH and depending on resources also a subset of HRP2+ and negatives (see Appendix 1: supplemental data), and which to discard.
 - a. Based on the number of discordant RDT or RDT-microscopy results, one can calculate the proportion of *P. falciparum* cases with false-negative HRP2 RDT results (indicating potential *pfhrp2/3* gene deletion) in the health facility or province, using the formula below.

$$\text{Proportion of } P. falciparum \text{ cases with suspected } pfhrp2/3 \text{ deletions} = \frac{\text{\# of Pf cases with discordant RDT or RDT-microscopy results}}{\text{\# confirmed } P. falciparum \text{ cases (by either RDT or microscopy)}}$$

7. Package and ship the DBS (minimum 2) for *P. falciparum* confirmatory testing and molecular +/- serological analysis for *pfhrp2/3* deletions to a WHO collaborating reference laboratory. Samples should be shipped under a Material Transfer Agreement (MTA) and ideally one DBS will remain in survey country reference lab at all times.

- a. Molecular-based confirmation of *pfhrp2/3* deletions as the cause of false-negative RDTs is needed to ensure that discordant results are not due to reasons other than *pfhrp2/3* gene deletions. Such reasons include operator error, false-positive pf-pLDH test lines or false positive microscopy results, or samples at the limit of detection of the RDTs, which may sometimes react sufficiently to generate a positive test line but other times may not. The contribution of these alternative causes of discordant results will vary.
 - b. Serological confirmation of *pfhrp2/3* deletions may also be performed using immunoassays, especially on samples where there is lack of agreement between RDT and PCR results.
8. Once the true number of cases of *pfhrp2/3* deletions causing false-negative HRP2 RDTs is known, then, for each sampling domain eg. province, the primary study outcome can be calculated

$$\begin{array}{lcl} \text{Proportion of } P. \textit{falciparum} \text{ cases} & & \# \text{ of confirmed } P. \textit{falciparum} \text{ patients with } pfhrp2/3 \text{ gene} \\ \text{with false-negative HRP2 RDT} & = & \text{deletions and HRP2 RDT negative results} \\ \text{results due to } pfhrp2/3 \text{ deletions} & & \# \text{ confirmed } P. \textit{falciparum} \text{ cases (by either RDT or} \\ & & \text{microscopy)} \end{array}$$

9. The statistical analysis of the proportion calculated in step 8 above will include the calculation of the corresponding 95% CI results. The analysis will result in one of three outcomes per province:
- a. **Outcome 1:** The estimated proportion is lower than 5% and the upper limit of the 95% CI is below 5%. In this case there is a high statistical confidence that the proportion of parasites with *pfhrp2/3* deletion causing false-negative RDT results within symptomatic patients is below 5%.
 - b. **Outcome 2:** The estimated proportion is higher than 5% and the lower limit of the 95% CI is above 5%. This result means that there is a high statistical confidence that the proportion of *pfhrp2/3* deletion causing false-negative RDT results in symptomatic Pf patients is greater than 5%.
 - c. **Outcome 3:** The statistical analysis shows that it is inconclusive (5% contained within the 95% CI) as to whether or not the prevalence of *pfhrp2/3* deletion causing false-negative RDT results in symptomatic Pf patients is greater than or less than 5%.
10. If outcome 2 is obtained, *pfhrp2* deletions are found to be prevalent (lower 95% CI is > 5%) in any province, the country programmes should make a nationwide switch to RDTs that do not rely exclusively on HRP2 for detecting *P. falciparum*, prioritized on the basis of the prevalence of *pfhrp2* deletions across provinces.
- a. A threshold of 5% was selected because it is somewhere around this point that the proportion of cases missed by HRP2 RDTs due to non-hrp2 expression may be greater than the proportion of cases that would be missed by less-sensitive pLDH-based RDTs.

- b. A nationwide change is suggested because mathematical models show parasites lacking *pfhrp2* genes will spread⁴ under HRP2-only RDT pressure and because the use of multiple RDTs in a country can complicate procurement and training practices.
- 11. If outcome 1 is obtained in all provinces, this country is recommended to establish a monitoring mechanism whereby this study is repeated in two years.
- 12. If outcome 3 is obtained in one or more provinces, this country has a few options depending on available resources:
 - a. establish a monitoring mechanism whereby this survey is repeated in two years (same as Outcome 1) or
 - b. repeat the survey in one year
 - c. continue screening patients to achieve larger sample size which will allow for a more accurate measurement of the true prevalence of *pfhrp2* deletion. Table 2 provides the sample sizes for determining if the true *pfhrp2* deletion prevalence is above or below the 5% threshold at the survey domain (eg. province) level.
- 13. Once the results of the survey are finalized and reported. Only the DBS from consenting individuals denoted on the DBS itself, the ziplock bag and case report form, should be kept for long term storage, all other materials associated with the survey (RDTs microscopy slides and DBSs) should be discarded. Consent forms linking patient names to survey IDs should be maintained by the Ministry of Health, the biobank holder should not itself possess the information linking patients' IDs to samples.

14. Survey staff

All activities should be under the supervision of the principal investigator. A dedicated study coordinator should be identified. It is recommended that there be a minimum of one supervisor per province where the survey is to be undertaken. Within each selected survey health facility, one to two survey staff should be trained to record the results of malaria diagnostic testing for all suspected malaria cases, enroll survey participants for long term sample storage, according to the protocol, collect RDTs +/- microscopy slides, collect DBSs on filter paper, conduct the questionnaire interviews, and properly store and package all samples/report forms for shipment to the laboratory. Either the supervisor or person with the requisite expertise should manage the data after collection, create indicator variables and analyse the data. Ideally, this should be done at the central level.

⁴ Gattton ML, Dunn J, Chaudhry A, Ciketic S, Cunningham J, Cheng Q. Use of PfHRP2-only RDTs rapidly selects for PfHRP2-negative parasites, with serious implications for malaria case management and control. J Infect Dis. 2017. doi: 10.1093/infdis/jix094.

TABLE 2:

Sample sizes for determining if the true *pfhrp2* deletion prevalence is above or below the 5% threshold at the survey domain (province) level

Percentage of confirmed <i>pfhrp2</i> deletions causing false negative HRP2 RDT results	Minimum number of individuals with confirmed <i>P. falciparum</i> infection to include per domain, to estimate sample size needed to ensure the 95% confidence interval (1-tailed test) does not include 5% prevalence of <i>pfhrp2/3</i> deletions (with design effect = 1.5)
%	n
2.6	268
2.8	343
3.0	445
3.2	584
3.4	783
3.6	1,080
3.8	1,550
4.0	2,350
4.2	3,829
4.4	7,116
4.6	16,701
4.8	69,557
5.0	-
5.2	75,037
5.4	19,441
5.6	8,942
5.8	5,199
6.0	3,445
6.2	2,463
6.4	1,865
6.6	1,470
6.8	1,194
7.0	993
7.2	843
7.4	726
7.6	635
7.8	560
8.0	500
8.2	449

TABLE 3.

Summary of test result combinations and limitations of the approach including only individuals with discordant HRP2 RDT results (positive by pf-pLDH or microscopy AND negative by HRP2 RDT)

HRP2 RDT	pf-pLDH RDT or microscopy	Molecular analysis performed	Interpretation of results and limitations in detecting <i>pfhrp2/3</i> deletions
+	+	No	<ul style="list-style-type: none"> • May be infection with <i>pfhrp2</i> deletion but HRP3 was detected by HRP2 RDT • May be multiclonal infection with parasites with and without <i>pfhrp2/3</i> deletion
+	-	No	<ul style="list-style-type: none"> • False-positive HRP2 RDT (or persisting HRP2 after resolution of infection) • May be infection with <i>pfhrp2</i> deletion but HRP3 was detected by HRP2 RDT or may be multiclonal infection with parasites with and without <i>pfhrp2/3</i> deletion AND <ul style="list-style-type: none"> • Low parasite density at or below the limit of detection of pf-pLDH RDTs and/or microscopy
-	+	Yes	<ul style="list-style-type: none"> • False-positive pf-pLDH RDT or microscopy • Low parasite density at limit of detection of RDTs (variable reactivity of test lines)
-	-	No	<ul style="list-style-type: none"> • Cannot exclude low-density infection missed by both RDTs, with undetected <i>pfhrp2/3</i> deletion

4.7 Sample storage

All RDTs and microscopy slides from consenting suspected malaria cases, labelled only with survey ID, should be stored in gas impermeable plastic bags until the survey and data analysis is completed. This is in case they are needed to resolve data inconsistencies or for additional DNA material. **Once the survey report is completed these should be discarded.**

The DBS on filter paper labelled with survey ID and tick box or label (No LTS (long term storage) or No BB (biobanking)) will be stored in an impermeable plastic bag with the same label and with the desiccant taken from the RDT package at ambient temperature at the health facilities until they are transported to the central reference laboratory. There, they will be sorted for onward molecular (including DNA sequencing) +/- serological analysis at a qualified participating laboratory (contact malaria_rdt@who.int for links to specific laboratories). After the survey results have been analysed and reported, any unused DBS or blood or DNA remaining on DBS after molecular testing and immunoassays should be discarded **unless patient consent/assent was granted**. For long term storage, DBS should ideally be frozen at -20°C or -70°C.

4.7.1 Long term storage of dried blood spots: biobank

As *pfhrp2/3* gene deletions are an emerging issue, little is known about the etiology, trends and associated genetic mutations that may confer survival advantages or disadvantages. New tools are needed to detect these deleted parasites and the availability of *pfhrp2/3* gene deleted parasite material could accelerate product development and evaluation. For these reasons, the biological

material collected during the survey could be used to advance future research and therefore consent for long term storage/biobanking of left-over biological material is requested.

At the time of consenting, all patients will be given an information sheet that is labelled with a unique survey ID and contact information for the study principal investigator and the National Malaria Reference Laboratory. They can contact these individuals to request removal of their materials from the biobank. If they lose their information sheet and unique survey ID, then their samples can be traced only by the Principal Investigator who maintains password protected access to the signed consent forms and corresponding survey IDs.

Long term storage requires that those coordinating the survey indicate how they envisage the materials being used in the future; to whom the materials will be entrusted and how confidentiality will be maintained eg. the Ministry of Health, research institute; how the quality of the material will be controlled; how the donor's authorization can be retracted and in which circumstances donors may need to be re-contacted. These requirements are laid out in Chapter 11 of the [International Ethical Guidelines for Health-related Research Involving Humans](#) Composition of supervision and field teams

4.8 Data storage and management

Survey case report forms and survey tally sheets do not include any unique/identifiable information. Patient names are only included on consents and assents and will be kept in a locked area during and after the study so that only the enrolling clinician, study coordinator and principal investigator ever have access. Electronic data should be password protected, double entered both at a provincial- or central-level facility. Software will be used for data management and analysis using Coding guides for all study variables

Once double data entries have been compared and any errors reconciled, data will be cleaned on an ongoing basis. All data will be collected using unique identification numbers linking the epidemiological and laboratory data and maintained in secure, password-protected files. During the survey, and at the central coordinating centre, all paper records collected will be stored in a secure location under lock and key.

The data are broadly classified as individual patient data, malaria infection data, laboratory data, and consent data. Other than on the consent form, there is no possibility to link survey IDs to patient name or any unique identifying characteristics. Encryption will be required for all tablets or electronic data capture devices used for data collection purposes. Permission will be required for data reuse. On-site data managers and their assistants will be trained in all data entry and management processes, and their training logs will be maintained and archived for data quality assurance checks

All health provider staff at survey sites should participate in training on the conduct of survey data collection. Personnel will be trained in the importance of maintaining consistency in the patient recruitment and data collection protocols and procedures.

The quality assurance approach will focus on providing support for the selection of survey subjects and survey sites, data collection, and management procedures. Data verification techniques will include logic, range and consistency checks. Data validation will be implemented via electronic data entry mechanisms, such as input masks, conditional logic and validation rules. Surveillance personnel will be trained on the rationale and importance of the data verification and validation processes, using specific examples to describe potential implications for the study results. Intermediate statistical analyses will serve as detective and corrective controls by identifying changes in enrolment rates, protocol deviations, duplication of data entry values, or incorrect data values. These results will be communicated to all key personnel on a weekly basis for as long as the cross-sectional data

collection is underway. Keeping both paper and electronic data will also serve as a secondary check for the accuracy of data.

4.9 Laboratory analyses

4.9.1 Rapid diagnostic tests

Rapid diagnostic tests will be performed according to manufacturers instructions

4.9.2 Molecular characterization

Molecular characterization should be conducted at a laboratory that has experience in malaria molecular +/- serological techniques and subscribes to the WHO external quality assessment (EQA) scheme for malaria nucleic acid amplification testing (NAAT) or other scheme for malaria molecular methods. Quantitative PCR is preferred over non-quantitative nucleic acid amplification methods, especially if parasite density is not being measured by microscopy.

The methods proposed below are based on Cheng et al. but may change depending on the reference laboratory [4].

4.9.2.1 DNA extraction and quality control

For verification of the DNA quality, an aliquot of the DNA should be used for amplification of the *msp1*, *msp2* and *glurp* genes, according to standard published protocols [18].

4.9.2.2 Molecular species diagnosis

Specific primer pairs should be used for four or five separate and specific amplification reactions of *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*.

4.9.2.3 Characterization of *pfhrp2* and *pfhrp3* sequences and gene deletions in samples

Suggested primer sequences, PCR conditions and expected amplification product sizes have been published [4]. *Pfhrp2* and *pfhrp3* genes should be characterized by amplification of two gene segments. One segment extends from the end of exon 1 to the start of exon 2, including the intron of each gene. The other segment consists of the entire exon 2, which codes for the histidine-alanine-rich repeat region of each protein. PCR assays should include appropriate controls, including DNA from lab strains with known deletions, such as DD2 (*pfhrp2*-deleted) and HB3 (*pfhrp3*-deleted). If the *pfhrp2* gene can be amplified, the sequence of the exon 2 amplicon will be determined and translated into an amino-acid sequence. This will enable the classification of the Pf HRP2 protein as type A, type B or type C/borderline structural group, according to the multiplied number of type 2 and type 7 repeats (see above). If the *pfhrp2* and/or *pfhrp3* genes cannot be amplified despite good quality of DNA (see 4.9.2) demonstrated by amplification of other single-copy gene sequences, it suggests that genes have been deleted. Optionally, in order to further confirm and characterize subtelomeric deletions, the following upstream and downstream flanking genes of *pfhrp2* and/or *pfhrp3* can be amplified: the HPC230 gene located ~5.5 kb upstream and HSP70 located ~6.5 kb downstream of *pfhrp2*; and the HPC475 gene located ~1.7 kb upstream and ACL located ~4.4 kb downstream of *pfhrp3*.

4.9.3 Serology

From DBS, ultrasensitive HRP2 and pLDH detection by multiplex bead immunoassay or ELISA may be used to support genotyping results and particularly to resolve discordance between RDT and PCR results.

4.10 Data analysis procedures

Following double data entry (see section 4.8) and reconciliation of any errors, the prevalence of false-negative HRP2 RDT results (diagnostic-based) that are suspected to be caused by *pfhrp2/3* gene deletions (output indicator 1) will be determined at the domain/provincial level, with 95% CIs estimated for all point estimates. This process should follow the tabulation format in the “dummy” table provided (Appendix 6). Provincial-level estimates of output 1 will then be disaggregated by age group, sex, village, and recent antimalarial treatment in order to see whether any patterns emerge. If desired, point estimates and 95% CIs can be weighted by relative facility size or patient flows. Differences between point estimates across sociodemographic or other collected variables can be investigated using χ^2 and/or logistic regressions, as desired.

After completion of the laboratory analyses, the prevalence of *pfhrp2/3* gene deletions based on genotyping +/- serology (will be estimated in the suspect cohort (output indicator 2) at the provincial and national levels (output indicator 3), with 95% CIs for all point estimates. This process should follow the tabulation format provided in the “dummy” table provided (Appendix 6). Output 3 will be disaggregated by province, age group, sex, village, and recent antimalarial treatment in order to see whether any patterns emerge. If desired, point estimates and 95% CIs can be weighted by relative facility size or patient flows, as well as by relative province size for national-level estimates. Differences between point estimates across sociodemographic or other collected variables can be investigated using χ^2 and/or logistic regressions, as desired.

Additionally, the final analysis will include:

- The total number suspected malaria cases screened
- The RDT positivity rate per health facility
- The comparative performance of RDTs and RDT test lines for the detection of *P. falciparum*

4.11 Dissemination of results

At the end of the study, the principal investigator will submit a report on the study and its main outcome. This report will be shared with the national malaria control programme and the Ministry of Health and will allow to formulate recommendations and to enable the Ministry of Health to make informed decisions about whether the current guidelines should be updated. The data will also be shared with WHO Global Malaria Programme so that it can be included in the Malaria Threat Maps (<https://apps.who.int/malaria/maps/threats/>) and the *World malaria report*.

- Indicate if the study will be presented during a scientific meeting or published.
- Indicate how the results will be disseminated to the study patients.
- If the study is community-based, mention how the community will be informed and how it is planned to maintain community participation.

5. Study timeline

It should be noted that the amount of time it will take to enrol the desired number of survey participants will depend on: 1) the number of suspected malaria cases seen each week at each facility; 2) the test positivity rate (i.e., number of positives per suspected malaria case) at each facility; and 3) the sample size needed to detect whether the observed prevalence of false-negative HRP2 RDT results caused by *pfhrp2/3* gene deletions is above or below the 5% threshold (see section 4.4). Prior to implementing this protocol, the NMCP and surveillance teams should assess the

expected time for enrolling the desired number of respondents within each province and plan accordingly. An example timeline is presented in Table 4; note that the start month will depend on the local transmission context.

TABLE 4.
Illustrative survey timeline

Activities	Year									
	J	F	M	A	M	J	J	A	S	O
IRB approval										
Sampling frame development										
Selection of provinces and facilities										
Procurement of all survey supplies										
Community engagement (as needed)										
Recruit and train data collectors										
Data collection at facilities										
Data entry										
Laboratory analysis										
Data analysis										
Presentation of findings										

6. Human subjects

6.1 Overview

All investigators will be trained in the ethical conduct of human research, the study objectives, methods of effective communication with study participants, and collection of high-quality data. The importance of informed consent and how to administer consent forms will be emphasized, and the study team will receive additional training specific to the tasks they will perform (e.g., interview techniques, sample collection and data confidentiality).

Prior to fieldwork being conducted, the relevant ethics committees/institutional review boards will be presented with all of the necessary documentation, including report forms, proposed procedures to minimize risk in the process of data collection, and consent forms and data management plans to ensure the confidentiality and safety of data.

All research participants will be asked to provide individual consent (or assent depending on the age of majority and federal guidelines in the country of enrolment) for their participation. Consent for long term storage/ biobanking is separate from consent to participate in the survey. In all cases, consent is voluntary, and participants have the right to refuse or withdraw at any time.

Informed consent will be obtained both verbally and in writing from all participants in the preferred local language (Appendix 3). As part of the consent process, the survey and biobanking will be explained and the consent form will be read to each person or given to participants to read

themselves. Participants will be asked questions to ensure their comprehension. It will be emphasized that participation is voluntary, and that participants have the right to withdraw consent at any time and the right to refuse to answer any question. The consent form will detail the design of the survey and biobank and analyses to be done, including a description of data storage. If participants agree, they will be asked to sign the consent or assent form or if illiterate, provide a thumbprint in conjunction with the signature of an independent witness, depending on national guidelines.

For children under the legal age, consent will be obtained from at least one parent or guardian; this is sufficient given the minimal risk posed. In addition, child assent (Appendix 4) will be obtained for children depending on the age of majority and federal guidelines in the country of enrolment, in addition to the consent of a parent or guardian. Children providing assent will be asked to sign next to their name or provide a thumbprint accompanied by the signature of an independent witness on the assent form. In cases where subjects under 18 years of age are considered “mature minors” (sometimes defined as pregnant, married, or otherwise the head of their household, depending on the country-specific context) and are able to provide consent for themselves, assent will not be sought. Examples of consent and assent forms are included in Appendices 3 and 4. The reading level of the consent form should be no higher than primary school level 8. All interviewers will be trained extensively in the consent procedure, and each form will be co-signed (or verified by their mark) by a team member in order to ensure that all participants have consented. A copy of the consent information sheet will be given to each subject and the certificate of consent maintained by the survey team. The names of the investigators will be included on all consent forms, with phone numbers and addresses for the participants to use if they have any questions or if they wish to withdraw their samples from the biobank, in the future.

6.2 Risks to human subjects

This surveillance and biobanking activity is of minimal risk to participants. The amount of blood collected is very small (~100–200µl), and participants may experience only a small bruise at the site from which blood is collected. The initial prick may lead to minor temporary discomfort or pain. Trained personnel will perform finger pricks in order to ensure that they are done in as safe a manner as possible. Precautions will be taken to avoid bleeding by applying cotton wool and pressure immediately to the prick site. Risk of infection will be minimized by cleaning the finger with an alcohol swab prior to pricking and using disposable lancets – one for each individual in order to avoid cross-contamination/transmission of infectious agents. Any concerns about potential risks will be mitigated as much as possible eg. through community sensitization prior to the survey.

6.3 Protection against risk

The surveillance and biobanking data collected is not considered to be of a sensitive nature. Therefore, there are minimal risks expected for the participant. Concerning confidentiality, only consenting patients will write their name on the consent form but this will not appear in any other registries/tally sheets, forms or diagnostic specimens associated with the survey or biobanking process. Steps will be taken to ensure that each study participant’s name will be protected. There is no linkage between clinic registries (which contain personal information) and survey report forms. DBS/Filter paper samples and other samples will be labelled using a survey ID only and consent for biobanking will be indicated directly on the DBS.

The proposed strategy to reduce any risks includes:

1. Explaining the physical procedures carefully to each participant so that they understand the potential pain associated with the collection of malaria data but also that the pain is most likely to be temporary.
2. Ensuring that health workers can answer commonly asked questions and understand the nature of the questions being asked.
3. Ensuring that health workers using RDTs in their routine work are observed for their competency in collecting and handling biological specimens and that all data entry personnel (these may also be the health workers) are trained in confidentiality, safety and informed consent procedures; all team members should be trained in universal precautions for handling biological specimens.
4. Training field supervisors in protocol management. Spot checks by the supervisory staff will provide further assessment of protocol management.
5. Using the most efficacious testing procedures available to ensure sterile and safe biological data collection and testing. The two RDTs and DBS will be collected simultaneously.
6. Assessing the practices for protecting against any blood-borne infections, including HIV, according to national guidelines, and corrective action plans should such infection occur from needle sticks during the collection of data. Training/retraining in the standard universal precautions (i.e., use of gloves and sterile equipment for all fluid transactions) will minimize the possibilities of transmission from participants to data collectors or vice versa. If a needle stick should occur, the recipient will immediately be offered appropriate counselling and treatment from the nearest relevant health facility according to national protocol.
7. Ensuring that the confidentiality procedures are designed to meet all contingencies in order to preserve the privacy of the participants.

6.4 Data monitoring and protection plan

Participants, parents and guardians will be informed that participating in a research study may involve a loss of privacy. All records will be kept as confidential as possible, and steps will be taken to ensure that each survey/biobanking participant's personal information will be protected. All long-term storage of personal data will be labelled with the participant's survey ID. Filter paper samples will be labelled using only a unique survey participant ID number, or barcode, which will only be linkable through the consent form (for consenting individuals), initially by the enrolling clinician and then later by study coordinator and principal investigator. For the laboratory analyses, there will be no link between the laboratory samples and the participants' identifiable information. All consent forms with survey IDs will be stored in locked cupboards and on password-protected computers accessible only to the study coordinator and principal investigator. . No individual identities will be used in any reports or publications resulting from the study.

6.5 Incentives

There will be no money or commodities offered as incentive for participation in the survey or biobanking.

7. References

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8. Appendices

- Appendix 1: Options for supplemental data collection
- Appendix 2: Survey facility tally sheet
- Appendix 3: Informed consent form template
- Appendix 4: Informed assent form template
- Appendix 5: Survey report form
- Appendix 6: Tabulation plan for prevalence of *pfhrp2/3* deletions

Appendix 1: Supplemental data analysis to determine the prevalence of *pfhrp2/3* gene deletions

To achieve the survey protocol primary output measures, only samples from patients with suspected *pfhrp2* deletions are prioritized for analysis for *pfhrp2/3* gene deletions. This approach reduces the number of patient samples that need to be transported and analysed by PCR +/- serology. However, as outlined in Table A1, there are limitations to this approach, as other malaria suspects with *pfhrp2/3* gene deletions will be missed. If after assessing the resources available to analyse the suspected *pfhrp2/3* deletions, there are funds available, additional analysis of a subset of DBSs' from HRP2 RDT positive and negative patients (missed by both RDTs or RDT and microscopy) can be conducted. The former, will identify infections caused by *pfhrp2* negative but *pfhrp3* positive parasites that still react with HRP2-based RDTs due to cross-reactivity between HRP2 and HRP3, as well as multiclonal infections with parasites with and without *pfhrp2/3* deletion. The latter will determine presence of *pfhrp2/3* deletions in very low density infections.

TABLE A1

Supplemental dried blood spot analysis options and associated limitations in detecting *pfhrp2/3* gene deletions

HRP2 RDT	pf-pLDH/ microscopy	Diagnosis	Order of priority for DBS analysis	Interpretation of results and limitations in detecting <i>pfhrp2/3</i> deletions
+	+	<i>P.falciparum</i>	2	<ul style="list-style-type: none"> • May be infection with <i>pfhrp2</i> deletion but HRP3 was detected by HRP2 RDT • May be multiclonal infection with parasites with and without <i>pfhrp2/3</i> deletion
+	-	<i>P.falciparum</i>	3	<ul style="list-style-type: none"> • False-positive HRP2 RDT (or persisting HRP2 after resolution of infection) • May be infection with <i>pfhrp2</i> deletion but HRP3 was detected by HRP2 RDT • May be a low-density Pf infection that does not result in pf-pLDH reaction with RDT due to low antigen concentration • May be multiclonal infection with parasites with and without <i>pfhrp2/3</i> deletion
-	+	<i>P.falciparum</i>	1	<ul style="list-style-type: none"> • False-positive pf-pLDH RDT or microscopy • Low parasite density at limit of detection of RDTs causing variable RDT reactivity
-	-	Negative for malaria	4	<ul style="list-style-type: none"> • Cannot exclude low-density infection missed by both RDTs, with undetected <i>pfhrp2/3</i> deletion. Use PCR to exclude malaria infection.

Appendix 2: *Pfhrp2/3* gene deletion survey facility tallysheet

This sheet will be filled out by all facilities. In each province, once 600 individuals with *P. falciparum* malaria have been seen (60 at each of 10 enrolment sites per province), calculate the proportion of discordant diagnoses (i.e. pf-pLDH or microscopy positive AND HRP2 RDT negative) among all positive *P. falciparum* diagnoses. When centrally compiled, section 4.6 points 6 and 8 can be used to interpret results and determine associated actions after statistical analysis of the molecular +/- serological confirmation data.

A	B	C	D	E	F	G
	Patient ID	Date of visit / test (DD/MM/YY)	Informed consent/assent	Pf case confirmed by at least one diagnostic method	Cumulative number of Pf positive cases (from column E)	Suspected false- neg HRP2 (pf-pLDH or microscopy positive AND HRP2 RDT negative)
1			Y / N	Y / N/ NA		Y / N/ NA
2			Y / N	Y / N/ NA		Y / N/ NA
3			Y / N	Y / N/ NA		Y / N/ NA
4			Y / N	Y / N/ NA		Y / N/ NA
5			Y / N	Y / N/ NA		Y / N/ NA

* Expand rows as needed

Total malaria suspects tested (column A) _____

Tally total after 60 *P. falciparum* cases detected per facility (column F – equals 37)

Number of suspected false-negative HRP2 RDT results (column G – sum of ‘yes’ responses)(a)

Total positive *P. falciparum* diagnoses by any test (last entry column F)

(b) _____

Percentage of all Pf cases with suspected false-negative HRP2 RDT results that need molecular+/- serological analysis for *pfhrp2/3* deletions (a / b)

Target: 60 *P. falciparum* cases detected per facility (last entry column F)

Appendix 3: Informed consent form (template)

(Note: the age of consent may differ between countries; as such, this form should be used in accordance with national guidelines and should be translated into the local language)

Name of proposal and version:

Survey ID: Label placed here

This informed consent form is for adults over age of majority years who attend name of site, who have been invited to participate in a survey for *pfhrp2/3* deletions and biobanking for future research

Name of principal investigator:

Name of organization:

Name of sponsor:

Survey sites:

This informed consent form has two parts:

- I. Information sheet (to share information about the study with you)
- II. Certificate of consent (for signatures if you agree to take part)

You will be given a copy of the information sheet

Part I: Information sheet

I am and I work with the National Malaria Control Programme.

Today you (or your child) are invited to participate in a research study to better understand if the malaria parasite is changing over time and affecting how well rapid diagnostic tests are working in this country. You (or your child) are being asked to participate in this study because you have presented with symptoms suggesting that you may have malaria.

This study has been reviewed by Local Institutional Review Board. No research activity will be conducted until you have had an opportunity to review this consent form, ask any questions you may have, and provide consent. We encourage you to ask questions now and at any time. If you decide to participate, you will be asked to sign the consent form or to provide a thumbprint in conjunction with the signature of an independent witness. A copy of this form will be provided to you. Your (or your child's) participation is completely voluntary and will in no way affect the treatment and care you receive for malaria or any other condition.

Why is this survey being done?

We are conducting this research survey because we want to look at samples of blood from people who we suspect could have malaria and then use the blood samples to see if the malaria parasite is changing over time and affecting the way malaria tests are working. A false-negative test result can mean that there is a problem with the test or that the malaria parasites have changed in a way that can make them hard to detect. We will ask you a few questions and perform some additional tests to determine if you have malaria. We will ask you if we can store your samples for future malaria research.

What are the study procedures? What will I be asked to do?

If you agree to take part in the survey, we will ask you basic questions such as your age, what village you live in, and the tests and medicines you have taken for malaria in the past few weeks. When we prick your finger to do the routine malaria rapid diagnostic test, we will also take a few extra drops to do an additional malaria test today and put drops onto paper for further testing to see whether the malaria parasites have changed in ways that make them hard to find. These tests will be carried out in (Name Location). There may be some leftover blood after we conclude the routine testing and survey and instead of discarding it, we would like your permission to store any such leftover blood at the (Name of Laboratory) for (indicate number of years, afterwards it will be destroyed. We may use it only for malaria-related studies in the future, particularly those that support the development of new diagnostic tests for malaria. Your materials will not be sold and use of them will only be authorized by a national and/or institutional research ethics committee.

What are the risks or inconveniences of the study?

There is very little risk of harm to anyone who agrees to participate in the survey. There may be a small bruise or temporary mild pain on the finger where the blood is taken. There is also a small chance of infection when blood is drawn. However, our careful procedures make this very unlikely. A possible inconvenience may be the additional 10-15 minutes added to your visit today, to complete the questions. This is a one-time survey and there will be no follow-up visits.

A second risk could be that someone outside the study team accesses your information; this is rare because we will not record your name (or your child's name) on any survey forms or your samples that are sent to the laboratory (s).

What are the benefits of the study?

The benefit of taking part in this study is that today we will do an extra test for malaria and this will improve the chances of finding malaria if it is in you. Also if you agree to donate your left-over blood, then your participation may result in public health programmes having better tests for malaria in the future and understanding if the malaria parasite is changing over time.

Are there costs to participate?

Participation is free of charge, but there is also no compensation to you (or your child) if you decide to take part in this study.

How will my personal information be protected?

We will make every effort to ensure that your information (or your child's information) is kept as confidential as possible. For example, we will not use your name or other identifying information on study documents, blood samples or in any publications; we will replace it with an identification number. Only those taking your consent today and the principal survey investigator will be able to link your name to your survey identification number. The consent forms bearing your name and signature will be kept stored in locked cupboards and on password-protected computers. The people responsible for the long-term storage of your (your child's) sample will not have your name.

Can I stop being in the study and what are my rights?

You do not have to participate in this survey, nor do you need to permit us to store your left-over samples for future research, if you do not want to, there will be no penalty to you. You can withdraw yourself (your child) from participating at any time without penalty and you (your child at age of majority) can also request that any left-over blood samples are withdrawn from long term storage.

For parents providing consent on behalf of children: If you do consent for long term storage of your child's leftover blood samples, then we advise you that when your child reaches maturity, that you give him/her the certificate of consent, which includes their survey ID number and contact information for the Principal Investigator, in case they wish to have those materials removed from storage and use.

Who do I contact if I have questions about the study?

If you have any questions you can contact the principal investigator, NAME at TELEPHONE NUMBER. If you have any questions about your rights as, or if you want to talk with someone who is not part of this research project, please contact NAME AND ADDRESS.

Survey ID: Label here

Part II: Certificate of consent

I have been invited to participate in a study that aims to better understand if the malaria parasite is changing over time and affecting how well rapid diagnostic tests are working in this country.

I have read the above information, or it has been read to me. I have had the opportunity to ask questions, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study.

Print name of participant:

Signature of participant:

Date:

dd/mm/yyyy

Long-term storage and future studies: I agree to allow the study team to store my (or my child's) (filter paper) blood sample for future studies on malaria. I understand that I can change my mind to not have my filter paper blood sample stored and used for future research.	If you agree, circle "YES," if you do not agree, circle 'NO'.	
	YES	NO

Adult/mature minor providing consent for self or child	Name	Signature/print	Date ____/____/____
--	------	-----------------	---------------------

Witness' signature: A witness' signature and the patient's thumbprint are required only if the patient is illiterate. In this case, a literate witness must sign. If possible, this person should be selected by the participant and should have no connection with the study team.

I have witnessed the accurate reading of the consent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of
witness:

and thumbprint of participant:

Signature of
witness:

Date:

dd/mmm/yyyy

<p><u>Long-term storage and future studies:</u> I have witnessed the accurate reading of the request for long term storage of samples, for future studies on malaria. I understand that the participant can change his/her mind to not have the filter paper blood sample stored and used for future research. The potential participant, who has had the opportunity to ask questions. I confirm that the participant agrees:</p>	If you agree, circle "YES," if you do not agree, circle 'NO'.	
	YES	NO

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, who has had the opportunity to ask questions. I confirm that the participant has given consent freely.

Print name of investigator:

Signature of investigator:

Date:

dd/mmm/yyyy

A copy of the information sheet with the survey ID affixed has been given to the patient. _____
(initials of the principal investigator or assistant).

Appendix 4: Informed Assent Form (template) for children between 7-15 years who are invited to participate in a survey for pfhrp2/3 deletions and biobanking for future

(Note: the age of assent may differ between countries; as such, this form should be used in accordance with national guidelines)

Name of proposal and version: _____
Survey ID: Label placed here _____

Name of principal investigator: _____
Name of organization: _____
Name of sponsor: _____
Survey sites: _____

This assent form has two parts:

- I. Information sheet (to share information about the study with you)
- II. Certificate of assent (for signatures if you agree to take part)

Part 1: Information Sheet

Introduction

My name is and my job is to do research to see if the malaria parasite is changing over time and affecting the way malaria tests are working.

I am going to give you information and invite you to be part of a research study. You can choose whether or not you want to participate. We have discussed this research with your parent(s)/guardian and they know that we are also asking for your agreement. If you are going to participate in the research, your parent(s)/guardian also have to agree. But if you do not wish to take part in the research, you do not have to, even if your parents agreed.

You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. You can decide whether to participate or not after you have talked it over. You do not have to decide immediately.

There may be some words you don't understand or things that you want me to explain more about because you are interested or concerned. Please ask me to stop at anytime and I will take time to explain).

Purpose: Why are you doing this research?

Sometimes malaria parasites can change over time and we want to be sure that the malaria tests we are using are working well – giving a positive results when malaria is present and a negative result when malaria is not present. In order to find out we have to test peoples blood using different kinds of malaria tests.

We also want your permission to save and store any of the leftover blood to use it to do more research to help us to make better tests for malaria and to understand how malaria is changing over time.

Choice of participants: Why are you asking me?

Children, like you, get sick more often than adults from malaria. Therefore it is really important to include children in this research.

Participation is voluntary: Do I have to do this?

You don't have to be in this research if you don't want to be. It's up to you. If you decide not to be in the research, its okay and nothing changes. This is still your clinic, everything stays the same as before. Even if you say "yes" now, you can change your mind later and it's still okay.

I have checked with the child and they understand that participation is voluntary __ (initial)

Procedures: What is going to happen to me?

Today we are going to test you for malaria just the same way as we do normally. However, we will collect a few extra drops of blood to do additional tests for malaria today and store some on a little piece of paper to do other tests later. We would then like to store any leftover blood to use it for research in the future.

I have checked with the child and they understand the procedures _____ (initial)

Discomforts: Will it hurt?

There will be a bit of discomfort when we prick your finger for blood but it will only last a few moments.

I have checked with the child and they understand the risks and discomforts _____ (initial)

Benefits: Is there anything good that happens to me?

By doing more than one test for malaria we have a better chance of finding it and getting you on the treatment. Also if you agree to donate your left-over blood, then your participation may someday result in your community having better tests for malaria in the future and understanding if the malaria parasite is changing over time.

I have checked with the child and they understand the benefits _____ (initial)

Reimbursements: Do I get anything for being in the research?

We do not offer any money or gifts for participating

Confidentiality: Is everybody going to know about this?

We will not tell other people that you are in this research and we won't share information about you to anyone who does not work in the research study.

Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key.

Sharing the Findings: Will you tell me the results?

As part of the research we are doing two tests for malaria on your blood at the same time today so we will let you and your parent/guardian know immediately the results of both of these tests. The other tests to look at the parasite will be done elsewhere in the country or abroad and these results will be shared with the government and other people like scientists to help them make decisions about the best test to use for malaria.

Right to Refuse or Withdraw: Can I choose not to be in the research? Can I change my mind?

You do not have to be in this research or you can agree to be part of this research but not allow us to store your leftover blood for future research. No one will be mad or disappointed with you if you say no. It's your choice. You can say "yes" now and change your mind later and it will still be okay.

Who to Contact: Who can I talk to or ask questions to?

You can ask me questions now or later. You can ask the nurse questions. I have written a number and address where you can reach us or, if you are nearby, you can come and see us. If you want to talk to someone else that you know like your teacher or doctor or auntie, that's okay too.

If you choose to be part of this research I will also give you a copy of this paper to keep for yourself. You can ask your parents to look after it if you want.

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

PART 2: Certificate of Assent

I understand this research is to understand if the malaria parasite is changing over time and affecting the way malaria tests work. I understand that I will get a finger prick for two malaria tests today and the a few drops on paper to use for other malaria research in the future.

I have read this information (or had the information read to me) I have had my questions answered and know that I can ask questions later if I have them.

I agree to take part in the research.

OR

I do not wish to take part in the research and I have not signed the assent below. _____
(initialed by child/minor)

Only if child assents:

Print name of child _____

Signature of child: _____

Date: _____

day/month/year

Long term storage

<u>Long-term storage and future studies:</u> I agree to allow the study team to store my blood sample for future studies on malaria. I understand that I can change my mind to not have my filter paper blood sample stored and used for future research.	If you agree, circle "YES," if you do not agree, circle 'NO'.	
	YES	NO

Only if child assents:

Print name of child _____

Signature of child: _____

Date: _____

day/month/year

If illiterate:

A literate witness must sign (if possible, this person should be selected by the participant, not be a parent, and should have no connection to the research team). Participants who are illiterate should include their thumb print as well.

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness (not a parent) _____ AND Thumb print of participant

Signature of witness _____

Date: _____

Day/month/year

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Print name of researcher _____

Signature of researcher _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the child understands that the following will be done:

- 1. finger prick for malaria test and blood spot on filter paper**

2. long term storage of the filter paper for future research

I confirm that the child was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this assent form has been provided to the participant.

Print Name of Researcher/person taking the assent _____

Signature of Researcher /person taking the assent _____

Date _____

Day/month/year

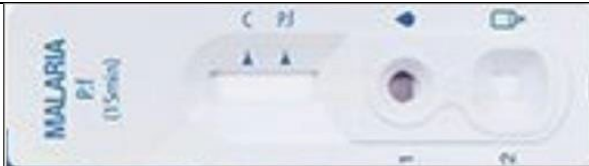
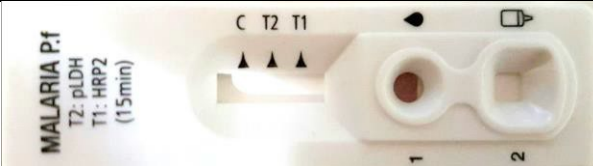
Copy provided to the participant _____ (initialed by researcher/assistant)

Parent/Guardian has signed an informed consent ____ Yes ____ No ____ (initialed by researcher/assistant)

Appendix 5: Survey case report form

Note: Each survey form should be prelabelled chronologically and there should be sufficient labels to place on RDTs, DBSs and plastic bags. Ideally the form should be produced in duplicate.

Forms should be pre-filled to indicate the health centre and RDT-specific information, i.e., name, product code, target antigens, etc., and sections that are not applicable (NA).

To be completed prior to participant interview											
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.	<p>Pre-entered for each health centre on printed form: RDT 1 (must include HRP2- National programme RDT)</p> <p>a. Name: b. Product code: c. Lot number: d. Expiry date: e. Target antigens:</p> <p>1. T1: 2. T2: 3. T3:</p>	 <table border="1" data-bbox="695 1010 1262 1234"> <tr> <td></td> <td>Box 1</td> </tr> <tr> <td>Control</td> <td>P.f HRP2</td> </tr> <tr> <td>+ / -</td> <td>+ / -</td> </tr> </table> <p>Circle correct result in each box above.</p> <p>Circle result of RDT: 1. Negative 2. <i>P. falciparum</i></p>		Box 1	Control	P.f HRP2	+ / -	+ / -			
	Box 1										
Control	P.f HRP2										
+ / -	+ / -										
6.	<p>RDT 2 (survey RDT)</p> <p>a. Name: b. Product code: c. Lot number: d. Expiry date: e. Target antigens:</p> <p>1. T1: 2. T2: 3. T3:</p>	 <table border="1" data-bbox="555 1626 1401 1850"> <tr> <td></td> <td>Box 2</td> <td></td> </tr> <tr> <td>Control</td> <td>T2 Pf-LDH</td> <td>T1 HRP2</td> </tr> <tr> <td>+ / -</td> <td>+ / -</td> <td>+ / -</td> </tr> </table> <p>Circle correct result in each box above.</p> <p>Circle result of RDT: 1. Negative 2. <i>P. falciparum</i></p>		Box 2		Control	T2 Pf-LDH	T1 HRP2	+ / -	+ / -	+ / -
	Box 2										
Control	T2 Pf-LDH	T1 HRP2									
+ / -	+ / -	+ / -									

7.	<p>a. Is RDT1 positive for <i>P.falciparum</i>? Y / N</p> <p>b. Is RDT2 positive for <i>P.falciparum</i>? Y / N</p> <p>If YES to EITHER question, provide treatment.</p>																				
8.	<p>DBS taken?</p> <p>a. Y / N</p> <p>b. consent for long term storage Y/N</p>																				
9.	<p>Microscopy</p> <table border="1"> <thead> <tr> <th>Microscopy</th> <th>Positive/Negative /NA</th> <th>Species</th> <th>Parasite count (parasites per microliter)</th> <th>Initials of microscopist</th> </tr> </thead> <tbody> <tr> <td>Field health facility</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>National Laboratory crosscheck (read 1)</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>National Laboratory crosscheck (read 2)</td> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table>	Microscopy	Positive/Negative /NA	Species	Parasite count (parasites per microliter)	Initials of microscopist	Field health facility					National Laboratory crosscheck (read 1)					National Laboratory crosscheck (read 2)				
Microscopy	Positive/Negative /NA	Species	Parasite count (parasites per microliter)	Initials of microscopist																	
Field health facility																					
National Laboratory crosscheck (read 1)																					
National Laboratory crosscheck (read 2)																					
<i>To be obtained from each malaria suspect</i>																					
10.	<p>Age in years</p> <p>_____</p>																				
11.	<p>Sex</p> <p>a. M</p> <p>b. F</p>																				
12.	<p>Village where malaria suspect resides</p> <p>_____</p> <p><i>From pre-populated list if possible</i></p>																				
13.	<p>In the past 2 weeks, have you had a test for malaria?</p> <p>a. No → Go to question 14</p> <p>b. Yes</p>																				
14.	<p>What was the result of the test ?</p> <p>a. Positive</p> <p>b. Negative</p> <p>c. Don't know</p>																				
15.	<p>In the past 2 weeks, have you taken any medicine for malaria?</p> <p>c. No → Go to question 17</p> <p>d. Yes</p>																				

16.	Antimalarial medicine taken	<i>From pre-populated list</i> d. ACT (whichever ACT is first-line drug in the country) e. Other ACTs (could be other names for first-line drug) f. Fansidar / SP / Sulfadoxine/pyrimethamine g. Quinine h. Panadol (antipyretics available in country) i. Other _____ j. Unknown k. NA
17.	Have you travelled to another locality of country in the past 30 days?	a. No → end b. Yes → go to question 18
18.	Where did you travel?	a. Country _____ b. Region _____ c. District _____ d. City/Village _____ e. NA Note: answers should be drop-down choices and place given for multiple entries
FOR SUPERVISOR USE ONLY		
S1	a. Is box 1 negative? b. Is box 2 positive?	Y / N Y / N If YES to part a and part b, the result is discordant .
REFERENCE LABORATORY USE ONLY		
17.	Molecular analysis	a. single copy gene 1 – present/absent/not done b. single copy gene 2 – present/absent/not done c. single copy gene 3 – present/absent/not done d. HRP2 Exon1 – present/absent/not done e. HRP2 Exon 2 – present/absent/not done f. HRP2 flanking 230 – present/absent/not done g. HRP2 flanking 228 – present/absent/not done h. HRP3 Exon 1 present/absent/not done i. HRP3 Exon 2 – present/absent/not done j. HRP3 flanking 485 – present/absent/not done k. HRP3 flanking 475 - present/absent/not done
18.	Serology	a. pfhrp2+/pan-LDH+ b. pfhrp2-/pan-LDH- c. pfhrp2+/pan-LDH- d. pfhrp2-/pan-LDH+

Appendix 6: Tabulation plan for prevalence of *pfhrp2/3* deletions^a

Characteristic	Suspected false-negative HRP2 RDT prevalence ^b (n=XX) (95% CI)	Confirmed <i>pfhrp2/3</i> deletion prevalence ^c (n=XX) (95% CI)
Age in years		
<2		
3–5		
6–9		
10–19		
20–29		
30–39		
40–49		
50–59		
≥60		
Sex		
Male		
Female		
Location		
Urban		
Rural		
Province (survey domain)		
Province 1		
Province 2		
Province 3		
Province 4		
Province 5		
Health facility (optional)		
Facility 1		
Facility 2		
Facility 3		
Facility 4		
Facility 5		
Facility 6		
Facility 7		
Facility 8		
Facility 9		
Facility 10		
Antimalarial treatment past 2 weeks		
Yes		
No		
Total		

a – Tabulations are based on *pfhrp2/3* deletion screening only in *P. falciparum* cases with discordant results. If all *Pf* cases or all suspects are screened for *pfhrp2/3* deletions, then this form should be revised accordingly.

b – Suspected false-negative HRP2-RDT *P. falciparum* prevalence = # discordant results (HRP2 negative & *pf*-pLDH or microscopy positive) / all *P. falciparum* cases confirmed by any diagnostic.

c – *pfhrp2/3* deletion prevalence = # *Pf* cases with *pfhrp2/3* deletion causing false-negative HRP2 RDT results / total # *P. falciparum* cases

