Title	**Surveillance of *plasmodium falciparum histidine rich protein 2/3 gene* deletions and biobanking to support future research of malaria parasites in Mainland Tanzania**
Study site(s)	A list of 100 sites from 10 regions (Dar es Salaam, Dodoma, Kagera, Kilimanjaro, Manyara, Mara, Mtwara, Njombe, Songwe and Tabora) is attached as Appendix 7
Protocol submission date	17/SEPT/2020
Protocol number	HRP2/VERSION 1.0 of 17th September 2020
Planned study dates	From September/2020 to October/2023

AUTHORSHIP ACKNOWLEDGEMENT:

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Deus S. Ishengoma[1,,2,3], Catherine Bakari[1], Misago D. Seth[1], Rashid A. Madebe[1], Celine I. Mandara[1], Beatus M. Lyimo[1,4], Filbert Francis[5], Daniel Mbwambo[6], Issa Garimo[6], Sijenunu Aaron[6], Abdallah Lusasi[6], Fabrizio Molteni[7], Ritha Njau[8], Jane A. Cunningham[9], Samwel Lazaro[6], Ally Mohamed[6], Jonathan J. Juliano[10], Jeffrey A. Bailey[11], Eric Rogier[12], Venkatachalam Udhayakumar[12]

1 National Institute for Medical Research, Dar es Salaam, Tanzania.
2 Faculty of Pharmaceutical Sciences, Monash University, Melbourne, Australia
3 Harvard T.H Chan School of Public Health, Boston, MA, USA
4 Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
5 National Institute for Medical Research, Tanga, Tanzania
6 National Malaria Control Programme, Dodoma, Tanzania
7 Swiss Tropical Public Health Institute, Dar es Salaam, Tanzania
8 World Health Organization, Country Office, Dar es Salaam, Tanzania
9 World Health Organization, Headquarters, Geneva, Switzerland
10 University of North Carolina, Chapel Hill, NC, USA
11 Brown University, Providence, RI, USA
12 Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA.

<u>Shortened citation for referencing:</u>

Ishengoma DS, et al. (2020). Study protocol: Surveillance of *plasmodium falciparum histidine rich protein 2/3 gene* deletions and biobanking to support future research of malaria parasites in Mainland Tanzania. Facilitated by MESA: https://mesamalaria.org/wp-content/uploads/2025/02/Surveillance-of-pfhrp-gene-deletions_MSMT-study-Tanzania.pdf

Project summary

Objectives

This survey is intended to determine whether the local prevalence of mutations in the *Plasmodium falciparum hrp2/3* genes causing false negative malaria rapid diagnostic tests (mRDTs) has reached a threshold that might require a regional or national change in diagnostic strategy. The specific objectives are to:

1. Measure the prevalence of suspected false-negative HRP2 mRDT results among symptomatic patients attending public health facilities with *P. falciparum* infection detected by a pf-pLDH mRDT;
2. Detect the parasite density and frequency of *pfhrp2/3* gene deletions in that cohort;
3. Determine the predictive value of false-negative HRP2 mRDT results for *pfhrp2/3* gene deletions in different settings;
4. Identify regions in which the prevalence of *pfhrp2/3* gene deletions causing false negative *P. falciparum* mRDTs is at or above 5%, warranting a change in mRDTs.

Secondary objectives:

1. To determine the diversity and population structure of the parasites from the surveyed regions
2. To determine the prevalence and frequency of drug resistance markers in the parasites
3. To establish a biobank of parasite samples for future research

The biobanking activity is intended to support future malaria epidemiological research and the development of new and/or improved health products particularly those targeting *pfhrp2/3* deleted parasites.

Survey site(s)	100 pre-selected public health facilities (from 10 regions) representing the spectrum of transmission and geographical diversity across the country
Target population	Individuals meeting case definition for suspected malaria
Survey type	Cross-sectional, multi-site
Primary output measures	1. Prevalence of suspected false-negative HRP2 mRDT results among symptomatic patients with *P. falciparum* malaria 2. Prevalence of *pfhrp2/3* gene deletions among symptomatic falciparum patients with a false-negative HRP2 mRDT result 3. Prevalence of *pfhrp2/3* gene deletions causing false-negative HRP2 mRDTs amongst all symptomatic *P. falciparum* confirmed cases.
Secondary output measures (optional)	1. Parasite species and density, as measured by serology and quantitative PCR in patients with suspected false-negative HRP2 mRDT results. 2. New knowledge concerning the epidemiology of *pfhrp2/3* deletions and other information e.g. genetic diversity and drug resistance status and new and improved tools to diagnose *pfhrp2/3* deleted parasites
Sample size	A sample size of 370 confirmed *P. falciparum* cases per region (sampling domain) from 10 health facilities in regions located in high transmission, moderate, low and very low transmission areas (37 per health facility) will be collected to quantify whether or not the prevalence of *pfhrp2* deletion is above 5%. Once the sample of 370 *P. falciparum* cases have been enrolled, molecular confirmation of *pfhrp2* deletions amongst suspected false-negative *P. falciparum* cases will be done.

Sampling method	In 10 pre-selected health facilities per region (sampling domain) at risk, a cross-sectional survey will measure the suspected and confirmed prevalence of *pfhrp2/3* gene deletions causing false-negative HRP2 mRDT results. A total of 37 *P. falciparum* confirmed cases will be included in each health facility.

Data collection

1. Ten regions (Dar es Salaam, Dodoma, Kagera, Kilimanjaro, Manyara, Mara, Mtwara, Njombe, Songwe and Tabora) will be included in the study.
2. Ten health facilities have been selected per region for testing. All selected health facilities have the capacity for malaria diagnosis using mRDTs.

   In the target population (suspected malaria cases), we will conduct routine case management procedures and obtain informed consent (or assent depending on the age of patient), to perform an additional mRDT and collect a dried blood spot (DBS) for laboratory analysis and for biobanking/long term storage to support future malaria epidemiological and diagnostic research
3. We will take clinical history including questions regarding age, sex, recent malaria diagnostic testing, antimalarial therapy and travel
4. Test all consenting individuals with suspected malaria simultaneously with the currently used HRP2 mRDT and a non-HRP2 method, using a pf-pLDH mRDT (separate single or multiple test line mRDT) in the health facility and collect minimum two DBS spots.
5. Record demographic and clinical history details and all test results on the survey report form.
6. Administer antimalarial therapy based on results from mRDT and according to national guidelines.
7. Retain used mRDTs for quality control and send minimum of two DBS spots from all consenting *P. falciparum* infected patients with negative HRP2 mRDT and positive pf-pLDH mRDT for molecular and serological analysis
8. Enrollment will stop once 370 individuals with confirmed *P. falciparum* malaria (37/site across the 10 health facilities in the regions), have been recorded in the survey tally sheet as having *P. falciparum*.

9. Ship all consent forms, tally sheets, survey report forms and patient samples to the central coordinating centre at NIMR in Dar es Salaam.
10. Central laboratory staff will review survey report forms and identify the suspected *pfhrp2/3* deletion cases and prioritize these DBS for molecular +/- serological analysis
11. Proceed with supplemental data analysis options as described in Appendix 1.
12. Store all mRDTs and DBS after survey results are finalized and reported, based on the donors' consent for long-term storage of DBS.

Statistical and analytic plan	The prevalence of suspected false-negative HRP2 mRDT results and *pfhrp2/3* gene deletions will be established at the regional level (sampling domain), with 95% confidence intervals (CI) estimated for all point estimates. If desired, point estimates and 95% CIs will be weighted according to relative facility size or patient flows. Differences between point estimates across sociodemographic characteristics and transmission levels, or other collected variables will be determined using X^2 and/or logistic regressions, as desired.
Reports, dissemination and publication	Periodic reports and a final report will be prepared and submitted to the Ministry of Health. The findings will be presented to different stakeholders at a dissemination meeting which will be held at the end of the study. The findings will also be shared with the World Health Organization (WHO) and other key partners. The results will be published in peer reviewed journals and presented at conferences locally and internationally.

1. Background and rationale

The recent reduction of malaria morbidity and mortality has been attributed to enhanced control including improved prompt diagnosis and effective treatment using rapid diagnostic tests (mRDTs) and artemisinin-based combination therapies (ACTs), respectively. The mRDTs currently used in most of the malaria endemic countries offer great potential for the immediate diagnosis of malaria infections. Rapid diagnosis of malaria allows for prompt treatment, especially in rural settings. The mRDTs used for confirmatory diagnosis of malaria are lateral flow immunochromatographic tests that detect *Plasmodium* parasite antigens in blood [1]. Three antigens are detected by current mRDTs: histidine rich protein 2 (HRP2), lactate dehydrogenase (LDH) and aldolase. HRP2 is an abundant protein expressed only by *Plasmodium falciparum* and is the target for the most commonly used mRDTs. 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 mRDTs tend to be more sensitive and heat-stable than mRDTs that detect LDH or aldolase [3].

While HRP2 mRDTs generally have the highest sensitivity of the mRDTs 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 mRDTs [4]. HRP2 mRDTs 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]. Even more recently, Beshir *et al* reported *pfhrp2* deletions in the neighbouring country of Kenya (in Kilifi and Mbita) [16] and sporadic occurrence has also been reported in some parts of Tanzania [17]. 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 mRDT results. Monitoring the accuracy of the mRDT results is thus critical. The main causes of false-negative mRDT 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]. Negative HRP2 mRDT results but pf-positive results by pf-pLDH mRDT 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 mRDT 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 regions 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 regions.

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 in neighbouring countries conduct surveillance for *pfhrp2/3* deletions, particularly amongst symptomatic patients [3]. To this end, the proposed study will be undertaken as part of the surveillance for clinically significant *pfhrp2/3* deletions in *P. falciparum*

in Mainland Tanzania, including all transmission zones. The study will also collect samples for long-term storage and biobanking to support future malaria research in this field. The findings of this study will enable the National Malaria Control Programme to map the distribution of these deletions, estimate the predictive value of suspected false-negative HRP2 mRDT 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 hrp*2/3 genes causing false-negative HRP2 mRDT results among symptomatic falciparum patients has reached a threshold that might require a national or subnational change in malaria mRDTs. 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 parasite species, density and frequency of *pfhrp2/3* gene deletions in that cohort.
3. Determine the predictive value of suspected false-negative HRP2 mRDT results for *pfhrp2/3* gene deletions in different settings.
4. Identify regions 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 mRDTs for detecting *P. falciparum*.
5. Additionally, malaria suspected patients will be asked to consent to long term storage (establishing a biobank) and use of DBS materials to: Support future malaria epidemiological research and product research and development for malaria.

3. Survey site(s) / target population

This survey will focus on individuals seeking treatment for mRDT-confirmed *P. falciparum* malaria at public health facilities. In Mainland Tanzania we propose to include 10 regions with varying malaria transmission intensity. At least 10 health facilities have been selected per region for testing from regions with high/moderate transmission regions of (Kagera, Mtwara, Mara and Tabora) and low/very low malaria transmission (Dar es Salaam, Dodoma, Kilimanjaro,

Manyara, Njombe and Songwe) [18]. All selected health facilities have the capacity for malaria diagnosis using mRDTs.

3.1. Inclusion criteria

- Patients of all age groups
- Meet case definition for suspected malaria case (fever in the last 48 hrs or fever at presentation and/or other malaria symptoms [19].
- Residence in the study area
- Providing informed consent

3.2. Exclusion criteria

- Previously enrolled in the survey
- Patients not residing in the study area
- Not providing informed consent
- Patients with severe malaria and other severe illnesses

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 the currently used mRDT (with HRP2 and panLDH; SD Bioline Pf/pan (Standard Diagnostic Inc., India), Care Start Malaria HRP2 (Pf/PAN, HRP2/pLDH; AccessBio Inc., NJ, USA), First Response (HRP2/pLDH Combo, Premier Medical Corp. India)) and an alternative method (i.e., pf-pLDH RDT) and collect a minimum of two DBS (spots). The frequency of suspected false-negative HRP2 mRDT results among symptomatic patients with *P. falciparum* malaria is the primary output (outcome 1). Molecular testing on the DBSs from suspected false negative HRP2 mRDT results will determine the prevalence of *pfhrp2/3* gene deletions in HRP2 mRDT 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 mRDT results (i.e., a negative HRP2 mRDT result but positive pf-pLDH and *P. falciparum* 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 mRDT result.
3. Prevalence of *pfhrp2/3* gene deletions causing false negative HRP2 mRDTs among all symptomatic *P. falciparum* confirmed cases.

The survey will identify the proportion of patients with suspected false-negative HRP2 mRDT results through routine diagnostic testing at health facilities using dual-method testing (the current HRP2 mRDT plus pf-LDH mRDT[1]). Molecular +/-serological testing to confirm *P. falciparum* infection and identify *pfhrp*2/3 deletions will initially be performed on DBSs collected from individuals with suspected false-negative HRP2 mRDT results but other samples will also be analysed. 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 mRDTs. In addition, there are several situations in which this indicator could miss a true *pfhrp2/3* gene deletion (see Table 2 below). First, individuals will not be detected if they have a low-density infection that is missed by pf-pLDH mRDT and also by HRP2 mRDT due to *pfhrp2/3* gene deletion. Second, HRP2 mRDT 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 co-infected with HRP2-expressing clones (multiple infections) 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.

[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)

4.3. Secondary output indicators

1. Parasite species and density, as measured by serology and quantitative PCR in patients with suspected false-negative HRP2 mRDT results; and in a subset of patients with negative results by both mRDTs.
2. Improved understanding of *pfhrp2/3* gene deletions and other information such as genetic diversity, relatedness, population structure and nation-wide profiles of drug resistance; 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 mRDT results caused by *pfhrp2/3* gene deletions at the regional level (survey domain) within Tanzania. 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%.

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

The 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 mRDT results caused by *pfhrp2/3* gene deletions is below 5% (signalling 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% (signalling that the observed level of *pfhrp2/3* gene deletion is above 5% with 95% confidence).

[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

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 mRDTs) within symptomatic patients with *P. falciparum* is below or above 5%, a sample size based on an expected population prevalence of 3.2% (n=370) per region (sampling domain) will be adequate. As a minimum, therefore, a sample of 370 individuals with a *P. falciparum* infection per region will be included (37 per health facility, 10 health facilities per region i) based on the formula above. Within the region, study health facilities were selected on the basis of probability proportional to size depending on 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 region when an estimate within 5% +/- 0.2 % is made, based on the formula above.

However, we will conduct the survey first with a sample of 370 per region, and molecular analysis will be undertaken first 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 region:

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 mRDT results within symptomatic *P. falciparum* 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 mRDT results in symptomatic *P. falciparum* 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* gene deletion causing false negative mRDT results in symptomatic *P. falciparum* patients is greater than or less than 5%.

4.5. Sampling

Stratified sampling was done to randomly select at least two regions in each of the four transmission zones/strata: high, moderate, low and very low [18]. In order to compensate and/or increase the number of samples from low/very low transmission areas, an additional region was selected making a total of three regions in each of the low and very low malaria transmission regions, respectively. In each region, a systematic random sample of a minimum of 10 health facilities was selected from a complete list of all facilities in each region. The facilities were stratified by type (hospitals, health centres and dispensaries) and included a measure of facility size depending on the number of fevers or suspected malaria outpatients seen at the health facility in a two-year period (2017 and 2018) from demographic health information system 2 (DHIS2). In the high/moderate malaria transmission regions, the number of febrile patients attended together with the number of patients with positive results by either mRDT and/or microscopy was used to determine the test positivity rates (TPR). The month (s) with the lowest TPRs at each health facility in 2017 and 2018 was/were used to generate the average TPR except for the regions with low/very malaria transmission. In these regions, the TPR for each facility was determined as above but the month(s) with the highest TPRs was/were used to generate the average TPR for the health facility. For each region, the TPRs of each health facility were used to calculate the average TPRs. The approach was used in the selection of facilities to account for the relative size of each facility, so that sampling is based on probability proportional to size (PPS). Selection of more than 10 health facilities per region was used to enable the team to recruit the required sample size; and this is expected to increase the estimated precision and decrease the length of time needed to meet enrolment targets.

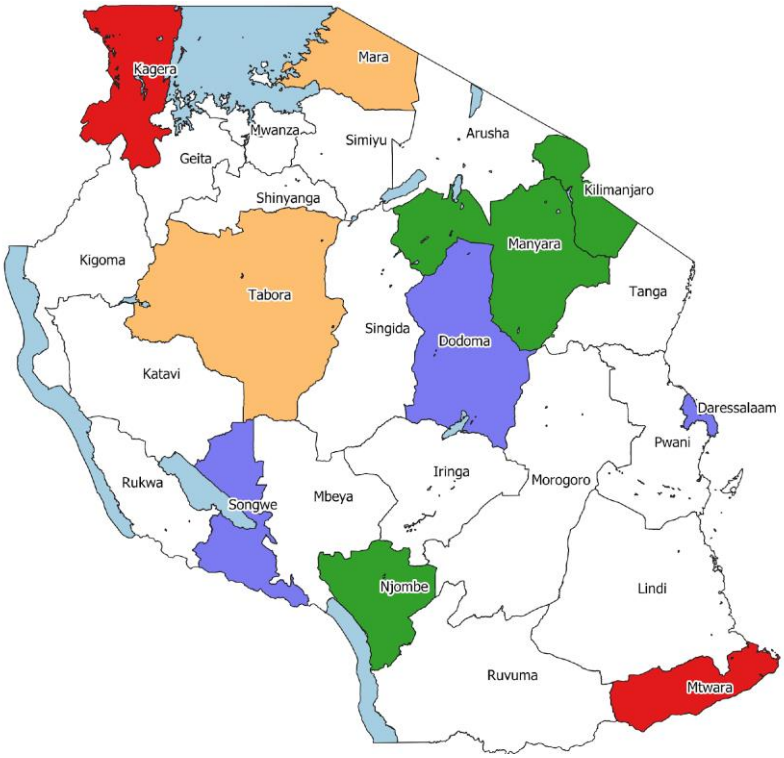

Figure 1: Map of the regions selected across epidemiological zones for the *pfhrp2/3* gene deletions survey.

4.6. Data collection and fieldwork

The following steps for data collection will be followed. *[Specific standard operating procedures (SOPs) for use by health facility staff will be developed before starting data collection]. The workflow is shown in figure 2:*

4.6.1. Ten regions were selected for the survey as described above. We used random selection using excel to select 10 out of 23 regions from Mainland Tanzania. We selected three regions each with very low (<1%) and low (1%), and two from areas with moderate (<10%) and high transmission (>10% prevalence in under-fives) were selected. Three regions of Kilimanjaro, Manyara and Njombe (very low), Dar es Salaam, Dodoma and Songwe (low), Mara and Tabora (moderate), and Kagera and Mtwara (high transmission) [18] were selected in each strata. 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. To increase the chance of detecting *pfhrp2/3* deletion in areas with high transmission, the survey will be conducted during the months with low TPRs as reported in 2017 and 2018.

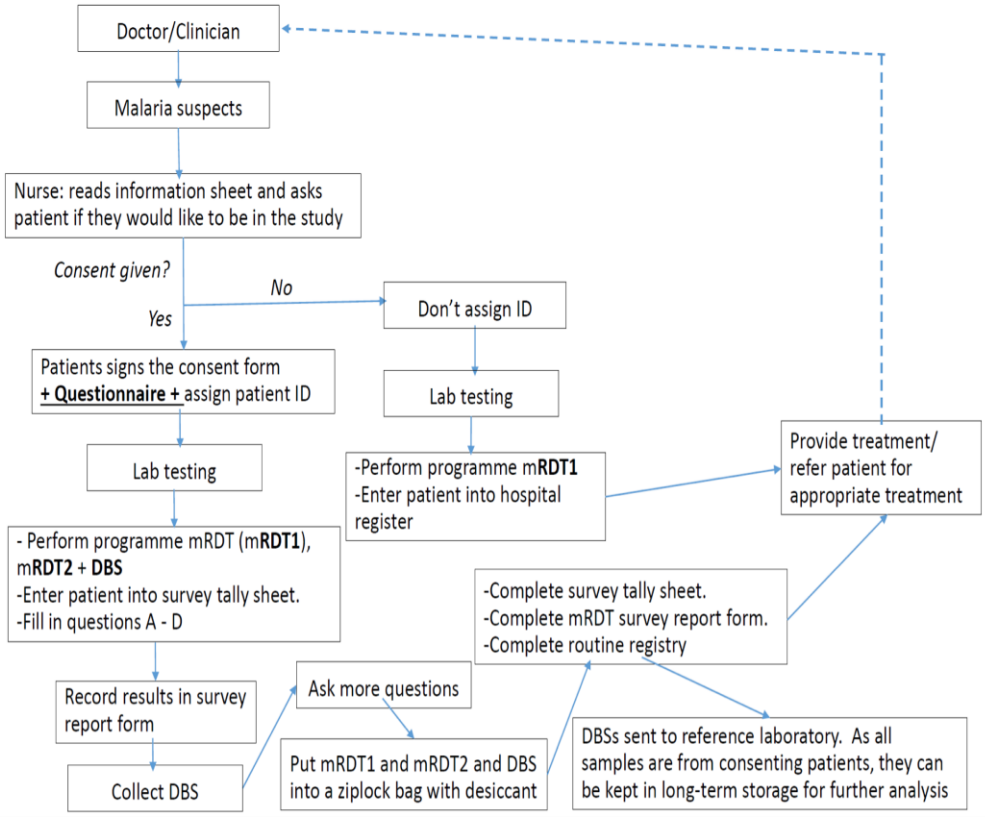

Figure 2: **Schematic presentation of workflow**

4.6.2. In each region, we selected 10 public health facilities making a total of 100 facilities in all regions.

- The number of facilities per region to be included took into account the expected mean number of suspected malaria patients seen in the facility each week and the mean TPR in the target area in order to ascertain the expected number of positives each week. The aim is to finish the fieldwork and collect a minimum sample size of 370 positives within an 8-week period. However, study duration has been adjusted depending on the actual number of cases expected to be sampled in each region.
- Health facilities for the sampling were selected from a complete list (sampling frame) of health facilities in each region (stratified by health facility type; dispensary, health centre and hospital), using systematic random sampling based on PPS [20] (and proportional to the size of the facility type strata in each region). The sampling frame included some estimate of facility size (fever or suspected malaria case load) and type (dispensary, health centre and hospital)

- Even though budgetary or logistical constraints may preclude the selection and inclusion of facilities using random sampling, we did not use a purposeful (or convenience) sample of facilities because we want the regional-level estimates of *pfhrp2/3* gene deletions to be statistically representative of the entire region.

4.6.3. Routine surveillance procedures

a. Patients will be triaged according to normal procedures. Any patient considered by the routine health provider e.g. physician, nurse, to be a suspected malaria case [19], according to national guidelines, will be asked for consent/assent (Appendix 4)
b. Only consenting individuals will sign their names 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 simultaneously tested according to manufacturers' instructions for use by two separate mRDTs, including the HRP2 RDT used by the national malaria control programme (NMCP) and a pf-pLDH mRDT, as well a minimum of two blood spots will be collected on filter paper. Non-consenting patients will receive routine care, no record of their name or identity will appear on any survey documents. Only consenting suspected malaria cases attending the facility will be sampled and a unique ID will be assigned on the survey tally sheet (Appendix 3 and 4).
d. All informed consent forms will be kept in a secure location under lock and key.
e. The current mRDT used in Tanzania is HRP2/pLDH based. This meets WHO procurement criteria and is approved for use in Tanzania. This is widely used by the NMCP for malaria diagnosis, as per national guidelines.

For the secondary diagnosis, pf-pLDH/HRP2 mRDT will be used for this study and it was selected because it meets WHO procurement criteria. At present, there are no mRDTs that meet the WHO performance criteria for detection of *P. falciparum* based on pf-LDH test line alone [21]; therefore, for the purposes of this survey, we selected pf-pLDH/HRP2-based mRDTs.

- For each consenting suspected malaria cases, the health provider will attach a unique survey ID, chronologically ordered, to a survey case report form (Appendix 5) using labels attached to the survey case report form. On the survey report form, the health worker will record answers to questions including age, sex, and recent history of malaria diagnostic testing, treatment and travel. Next, using the labels attached to the survey case report form with the same ID, the health worker (treating clinician or laboratory worker) will label the 2 different mRDTs and DBS. He/she will perform the tests, record the results on the survey case report form (Appendix 5, section 7) and inform patient of the 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 mRDT, as per national guidelines. Treatment will be provided based on a positive result from either mRDT as both are WHO prequalified. Patients with negative mRDT results will be managed as per national guidelines.

4.6.4. The study team will take precautions to prevent potential transmission of Covid-19 between health workers and patients. These will include use of masks, sanitizing, social distancing and proper disposal of wastes. Similar precautions will be taken during the training of the study teams.

a. All used mRDTs from each consenting suspected malaria cases will be stored until the survey is completed in a dry and protected area for survey quality control purposes.
b. The minimum of two DBSs (50µl per spot) on filter paper will be placed

- In a clean, dry and protected area, and allowed to dry for 3-4 hours.
- Once dry, the filter paper will be placed with the desiccant (from the mRDT package) in an impermeable plastic bag. The bag will be labelled with survey ID

c. Once the desired sample size of infected, consenting individuals is obtained at each facility, the survey case report forms and corresponding mRDTs and DBS will be compiled and collected by the regional supervisor who will send them to NIMR headquarters in Dar es Salaam (or any other central coordinating centre to be established). No names or other unique identifying information will be contained on forms, tally sheets, mRDTs or

DBSs. The only link between patient name and survey ID number is on the consent form of consenting individuals.

d. Upon receipt of forms/mRDTs/DBSs, the regional supervisor will review the report form mRDT results section (Appendix 2, section 7), determine which DBSs to prioritize for molecular +/- serological analysis, specifically, the HRP2 mRDT negative and pf-LDH positives (discordant samples) and depending on resources also a subset of HRP2 positives and negatives (see Appendix 1: supplemental data), and which to discard.

4.6.5. Based on the number of discordant mRDT results, we will calculate the proportion of *P. falciparum* cases with false-negative HRP2 mRDT results (indicating potential *pfhrp2/3* gene deletion) in the health facility or region, using the formula below.

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

4.6.6. The regional supervisor will package and ship DBS (minimum 2 per enrolled person) for *P. falciparum* confirmatory testing and molecular and serological analysis of *pfhrp2/3* deletions to a NIMR laboratory in Dar es Salaam and at the Centres for Disease Control and Prevention (CDC), Atlanta, USA. One DBS sample per enrolled person will be shipped to CDC under a Material Transfer Agreement (MTA) and one DBS will remain in Tanzania at the NIMR laboratory at all times.

a. Molecular-based confirmation of *pfhrp2/3* deletions as the cause of false-negative mRDTs 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 samples at the limit of detection of the mRDTs, 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 will also be performed using immunoassays, especially on samples where there is lack of agreement between mRDT and PCR results.

4.6.7. Once the true number of cases of *pfhrp2/3* deletions causing false negative HRP2 mRDTs is known, then, for each region, the primary study outcome will be calculated:

$$\text{Proportion of } P.\ falciparum \text{ cases with false-negative HRP2 mRDT results due to } pfhrp2/3 \text{ deletions} = \frac{\#\text{ of confirmed falciparum patients with } pfhrp2/3 \text{ gene deletions and HRP2 mRDT negative results}}{\#\text{ confirmed } P.\ falciparum \text{ cases (by either mRDT)}}$$

4.6.8. The statistical analysis of the proportion calculated in step 9 above will include the calculation of the corresponding 95% CI. The analysis will result in one of three outcomes per region:

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 mRDT 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 mRDT results in symptomatic *P. falciparum* 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 mRDT results in symptomatic *P. falciparum* patients is greater than or less than 5%.

4.6.9. If outcome 2 is obtained, *pfhrp2* deletions are found to be prevalent (lower 95% CI is > 5%) in any region, NMCP will make a nationwide switch to mRDTs that do not rely exclusively on HRP2 for detecting *P. falciparum,* prioritized on the basis of the prevalence of *pfhrp2* deletions across regions.

a. A threshold of 5% was selected because it is somewhere around this point that the proportion of cases missed by HRP2 mRDTs due to non-*pfhrp2*

gene expression may be greater than the proportion of cases that would be missed by less-sensitive pLDH-based mRDTs.

b. A nationwide change is suggested because mathematical models show parasites lacking *pfhrp2* genes will spread[3] under HRP2-only mRDT pressure and because the use of multiple mRDTs in a country can complicate procurement and training practices.

4.6.10. If outcome 1 is obtained in all regions, NMCP will establish a monitoring mechanism whereby this study will be repeated in two years.

4.6.11. If outcome 3 is obtained in one or more region, NMCP has a few options depending on available resources:

a. To establish a monitoring mechanism whereby this survey is repeated in two years (same as Outcome 1) or

b. To repeat the survey in one year

c. To continue screening patients to achieve larger sample size which will allow for a more accurate measurement of the true prevalence of *pfhrp2* deletion. Table 1 provides the sample sizes for determining if the true *pfhrp2* deletion prevalence is above or below the 5% threshold at the regional level.

4.6.12. Once the results of the survey are finalized and reported, only the DBS from consenting individuals will be kept for long term storage, all other materials associated with the survey (mRDTs and DBSs) will be discarded. Consent forms linking patient names to survey IDs will be maintained by NIMR, the biobank will be established at NIMR and shall not possess the information linking patients IDs to samples.

4.6.13. Survey staff

All activities will be under the supervision of the principal investigator. A dedicated Project manager will coordinate the field and laboratory activities. At the regional level, the regional malaria focal person, NMCP and NIMR investigators will train and supervise the health facility staff (2 per facility) and collect data and samples for quality control and onward shipment to NIMR laboratory.

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[3] Gatton 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 1: Sample sizes for determining if the true *pfhrp2* deletion prevalence is above or below the 5% threshold at the regional level

Percentage of confirmed *pfhrp2* deletions causing false negative HRP2 mRDT results	**Minimum number of individuals with confirmed *P. falciparum* infection to include per region, to estimate sample size needed to ensure the 95% confidence interval (1-tailed test) does not include 5% prevalence of *pfhrp2/3* deletions**
%	N
3.0	205
3.2	260
3.4	369
3.6	487
3.8	757
4.0	1,082
4.2	2,016
4.4	3,484
4.6	11,133
4.8	32,202
5.0	
5.2	34,739
5.4	10,240
5.6	4,379
5.8	2,457
6.0	1,590
6.2	1,123
6.4	841
6.6	658
6.8	531
7.0	459
7.2	386

7.4	331
7.6	287
7.8	253
8.0	224
8.2	205

TABLE 2. **Summary of test result combinations and limitations of the approach including only individuals with discordant HRP2 mRDT results (positive by pf-pLDH AND negative by HRP2 mRDT)**

HRP2 mRDT	pf-pLDH mRDT	Molecular analysis performed	Interpretation of results and limitations in detecting *pfhrp2/3* deletions
+	+	No	• May be infection with *pfhrp2* deletion but HRP3 was detected by HRP2 mRDT • May be multiclonal infection with parasites with and without *pfhrp2/3* deletion
+	-	No	• False-positive HRP2 mRDT (or persisting HRP2 after resolution of infection) • May be infection with *pfhrp2* deletion but HRP3 was detected by HRP2 mRDT or may be multiclonal infection with parasites with and without *pfhrp2/3* deletion AND • Low parasite density at or below the limit of detection of pf-pLDH mRDTs
-	+	Yes	• False-positive pf-pLDH mRDT • Low parasite density at limit of detection of mRDTs (variable reactivity of test lines)
-	-	No	• Cannot exclude low-density infection missed by both mRDTs, with undetected *pfhrp2/3* deletion

4.7. Sample storage

All mRDTs from all suspected malaria cases, labelled only with survey ID, will 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 will be stored if donors provide consent; otherwise, they will be discarded.

The DBS on filter paper labelled with survey ID will be stored in an impermeable plastic bag with the desiccant taken from the mRDT package at ambient temperature at the health facilities until they are transported to the NIMR laboratory. There, they will be sorted for molecular (including DNA

sequencing) and serological analysis at NIMR and CDC laboratory with the support of WHO. After the survey results have been analysed and reported, any unused DBS or blood or DNA remaining on DBS after molecular testing and immunoassays will be discarded unless patient consent/assent was granted. For long-term storage, DBS will be frozen at -20°C or -80°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 aetiology, 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 proposed survey will be used to advance future research and therefore consent for long-term storage/biobanking of left over biological material will be requested.
The materials from consenting patients, will be stored for long term uses at the NIMR Laboratory and maintained in properly monitored -20/80°C freezers.

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 NIMR 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.

Composition of supervision and field teams

There will be at least one supervisor per region where the survey will be undertaken. Within each selected health facility, two to three survey staff will be trained to record the results of malaria diagnostic testing for all suspected malaria cases, enrol survey participants for long term sample storage, according to the protocol, collect mRDTs, collect DBSs on filter paper, conduct the questionnaire interviews, and properly store and package all

samples/report forms for shipment to the NIMR laboratory. The supervisor will manage the data after collection from the health facilities and perform the first data entry while the data manager will supervise the second entry which will be done centrally at NIMR. The data manager will create indicator variables and analyse the data.

4.8. Data storage and management

Survey case report forms and survey tally sheets will not include any unique/identifiable information. Patient names will only be included on consents and assents and will be kept in locked areas during and after the study so that only the enrolling clinician, the project manager and principal investigator ever have access. Electronic data will be password protected, single entered at the regional level while the second entry will be done centrally at NIMR, and at the participating laboratory facilities, using electronic data entry screens. Survey case report forms and data entry will be developed and tested before the study. Coding guides will be developed for all study variables and will be used across sites to ensure comparability.

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 (NIMR), 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 patient name or any unique identifying characteristics to survey IDs. To safeguard data integrity, all survey/surveillance personnel will be required to use password-protected computers to access the data. Encryption will be required for all tablets or electronic data capture devices used for data collection purposes. Permission will be required for data reuse. The data manager and his/her 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 health facilities will 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. Survey 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. The data generated will be owned by NIMR and NMCP on behalf of the Government of Tanzania. Data transfer and sharing between collaborators and partners will be approved by the Project Advisory committee (PAC) which is responsible for overall management of the project. PAC is under the chairmanship of the Director of Preventive Services of the Ministry of Health.

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 will be conducted at NIMR and CDC laboratories that have experience in malaria molecular and serological techniques and subscribe to the WHO external quality assessment (EQA) scheme for malaria nucleic acid amplification testing (NAAT) or other scheme for malaria molecular methods. Quantitative PCR nucleic acid amplification methods will be used to determine parasite density. All DBS collected from patients with positive results by one or both mRDTs and a sub-sample of DBS with negative mRDT results will be analysed.

The methods proposed below are based on Cheng et al. and depending on the reference laboratory of CDC's validated procedures [4].

4.9.2.1. ***DNA extraction and quality control***

For verification of the DNA quality, an aliquot of the DNA will be used for amplification of the *msp1*, *msp2* and *glurp* genes, according to standard published protocols [22].

4.9.2.2. ***Molecular species diagnosis***

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

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 will 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 will include appropriate controls, including DNA from laboratory 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 PfHRP2 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 the genes have been deleted. In order to further confirm and characterize subtelomeric deletions, the following upstream and downstream flanking genes of *pfhrp2* and/or *pfhrp3* will be amplified: the HPC230 gene located ~5.5 kb upstream and HSP70 located ~6.5kb downstream of *pfhrp2*; and the HPC475 gene located ~1.7 kb upstream and ACL located ~4.4 kb downstream of *pfhrp3*.

4.9.2.4. ***Serology***

From DBS, ultrasensitive HRP2, Aldose and pLDH detection by multiplex bead immunoassay will be done and used to support genotyping results and particularly to resolve discordance between mRDT and PCR results. A protocol developed and optimized at CDC will be used for antigen assay. Briefly a 6mm punch of each sample will be taken and eluted in blocking buffer containing: PBS, 0.5% Polyvinyl alcohol (Sigma), 0.8% Polyvinylpyrrolidine (Sigma), 0.1% casein (ThermoFisher Scientific, Waltham, MA), 0.5% bovine serum albumen (BSA) (Sigma), 0.3% Tween-20, 0.05% sodium azide, and 0.01% *E. coli* extract to prevent non-specific binding. The filter bottom of the plate will be wetted with 100µL wash solution containing; phosphate buffered saline (PBS) with 0.05% Tween 20 (PBST). For each assay, a mix of the three coupled bead regions will be made in 5mL reagent diluent so that 800 beads of each region will be added per well in the assay plate (BioPlex Pro, BioRad, Hercules, CA). The beads will be washed twice, and 50µL of blood sample will be incubated with beads for 90 min under gentle shaking. After 3 washes, the beads will be incubated for 45 min with a 50uL mix of detection antibodies: anti-pAldolase (1:2000x, rabbit anti-aldolase, Abcam), anti-pLDH (1:500x of 1:1 mixture [mouse IgG anti-pLDH (Fitzgerald) and mouse anti-PvLDH (Fitzgerald)]), and anti-HRP2 (1:500x, of a 1:1 mixture of mouse IgG:IgM anti-HRP2, ICL labs). The plates will be washed three times, and subsequently

incubated with 50µL streptavidin-phycoerythrin (1:200x, Invitrogen, Carlsbad, CA). Thereafter, the plates will be washed three times, and after a final 30 min wash with reagent diluent, they will be subjected to one wash, re-suspended in 100µL PBS, and read on a MAGPIX instrument (Luminex Corp) by generating the median fluorescence intensity (MFI) signal for 50 beads in each unique region. The final measure, denoted as MFI-bg, will be reported by subtracting MFI values from beads on each plate only exposed to sample diluent during the sample incubation step.

4.10. Data analysis procedures

Following double data entry (see section 4.8) and reconciliation of any errors, the prevalence of false-negative HRP2 mRDT results (diagnostic-based) that are suspected to be caused by *pfhrp2/3* gene deletions (output indicator 1) will be determined at the regional level, with 95% CIs estimated for all point estimates. This process should follow the tabulation format in the "dummy" table provided (Appendix 6). Regional-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 will be weighted by relative facility size or patient flows. Differences between point estimates across sociodemographic or other collected variables will 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 regional and national levels (output indicator 3), with 95% CIs for all point estimates. This process will follow the tabulation format provided in the "dummy" table provided (Appendix 6). Output 3 will be disaggregated by region, age group, sex, village, and recent antimalarial treatment in order to see whether any patterns emerge. If desired, point estimates and 95% CIs will be weighted by relative facility size or patient flows, as well as by relative

regional size for national-level estimates. Differences between point estimates across sociodemographic or other collected variables will 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 mRDT positivity rate per health facility
- The comparative performance of mRDTs and mRDT test lines for the detection of *P.falciparum*

4.11. Stakeholders' engagement and Dissemination of results

Before the survey, meetings will be held with malaria stakeholders including members of the council and regional health management teams (CHMTs/RHMTs) from the regions where the survey will be undertaken. The meetings will be used to raise awareness about *pfhrp2/3* deletion and its impacts on malaria case management. 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 NMCP 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.

The findings of the study will be presented at scientific meetings and also published in peer reviewed journals. Meetings will be held with malaria stakeholders from the study regions to provide the feedback of the study including the results and future plans for surveillance of *pfhrp2/3* deletions in Tanzania. In these meetings, representatives will be invited from the study health facilities to attend the dissemination meetings which will be held in different zones of Tanzania. A summary of survey findings will be prepared in both English and Kiswahili and provided to health facilities and the district medical officers to allow further dissemination of the findings to study

participants and their communities. Community participation in the survey and future initiatives will be maintained through involvement of the CHMT/RHMT members from selected districts in the survey regions.

5. Survey timeline

The survey will be initially undertaken for 12 months but the amount of time it will take to enrol the desired number of survey participants at each health facility will depend on: 1) the number of suspected malaria cases seen each week at the facility; 2) the TPR (i.e., number of positives per suspected malaria case) at the facility; and 3) the sample size needed to detect whether the observed prevalence of false-negative HRP2 mRDT results caused by *pfhrp2/3* gene deletions is above or below the 5% threshold (see section 4.4). After a thorough assessment, the expected time for enrolling the desired number of respondents within each region will be determined. Based on availability of funding, the survey will be repeated after 2 years and the entire project will cover a period of 3.5 years . The timeline for this study is presented in Table 3; *(the start month of the survey will depend on the local transmission context in each region).*

TABLE 3. Timelines for the study

	ACTIVITIES (YEAR 1)	Jul-20	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21
1	IRB approval												
2	Finalize study tools												
3	Preparation of database												
4	Testing of study tools and database												
5	Sourcing/Procurement of supplies												
6	Selection and training of data collectors												
7	Data and sample collection												
8	Laboratory analysis in USA												
9	Lab set up in Tanzania												
10	Data analysis												
11	Preparation of reports												
	ACTIVITIES (YEAR 2)	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22
1	Lab set up in Tanzania												
2	Sourcing/Procurement of supplies												
3	Data analysis												
4	Preparation of reports												

5	Dissemination of findings												
6	Publication												
	ACTIVITIES (YEAR 3)	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23	May-23	Jun-23
1	IRB approval												
2	Sourcing/Procurement of supplies												
3	Training of data collectors												
4	Data and sample collection												
5	Laboratory analysis												
6	Data analysis												
7	Preparation of reports												
8	Publication												
	ACTIVITIES (YEAR 4)	Jul-23	Aug-23	Sep-23	Oct-23								
1	Data analysis												
2	Preparation of a final report												
3	Dissemination of findings												
4	Publication												

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, 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 will be presented to the Medical Research Coordinating Committee (MRCC) of the National Institute for Medical Research (NIMR). The protocol will be reviewed and ethical approval will be provided by MRCC of NIMR. The protocol will also be submitted for review and approval by the ethics committee of WHO. All research participants will be asked to provide individual consent (or assent depending on the age of study subjects and national guidelines) for their participation in the survey and future research.

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. The informed consent form will be translated in Kiswahili and consent will be obtained both verbally and in writing from all participants (Appendix 3&4). 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 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 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 4A&B) will be obtained for children over 6 but under 17 years of age, 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/parent/guardian) 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 (Standard 7). 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 survey and biobanking activity is of minimal risk to participants. The amount of blood to be 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 through community sensitization prior to the survey.

6.3. Protection against risk

The survey and biobanking data to be collected is not considered to be of a sensitive nature. Therefore, there are minimal risks expected for the participants. 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:

i. 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.
ii. Ensuring that health workers can answer commonly asked questions and understand the nature of the questions being asked.
iii. Ensuring that health workers using mRDTs in their routine work are observed for their competency in collecting and handling biological specimens and that all data entry personnel (including regional supervisors, data clerks and data manager) are trained in confidentiality, safety and informed consent procedures; all team members will be trained in universal precautions for handling biological specimens.
iv. Training of regional supervisors in protocol management. Spot checks by the supervisory staff will provide further assessment of protocol management.

v. Using the most efficacious testing procedures available to ensure sterile and safe biological data collection and testing. The two mRDTs and DBS will be collected simultaneously.
vi. 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.
vii. 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 Project Manager 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 Project manager 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. Budget (depends on funding available)

8. References

1. Howard RJ, Uni S, Aikawa M, Aley SB, Leech JH, Lew AM *et al*.: Secretion of a malarial histidine-rich protein (Pf HRP II) from Plasmodium falciparum-infected erythrocytes. *J Cell Biol* 1986, 103: 1269-1277.
2. Lee N, Baker J, Andrews KT, Gatton ML, Bell D, Cheng Q *et al*.: Effect of sequence variation in Plasmodium falciparum histidine- rich protein 2 on binding of specific monoclonal antibodies: Implications for rapid diagnostic tests for malaria. *J Clin Microbiol* 2006, 44: 2773-2778.
3. WHO. False-negative mRDT results and implications of new reports of P. falciparum histidine-rich protein 2/3 gene deletion. 2017. Geneva, Switzerland, World Health Organization.
4. Cheng Q, Gatton ML, Barnwell J, Chiodini P, McCarthy J, Bell D *et al*.: Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. *Malar J* 2014, 13: 283.
5. Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW *et al*.: A large proportion of P. falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLoS ONE* 2010, 5: e8091.
6. Bharti PK, Chandel HS, Ahmad A, Krishna S, Udhayakumar V, Singh N: Prevalence of pfhrp2 and/or pfhrp3 Gene Deletion in Plasmodium falciparum Population in Eight Highly Endemic States in India. *PLoS ONE* 2016, 11: e0157949.
7. Koita OA, Doumbo OK, Ouattara A, Tall LK, Konare A, Diakite M *et al*.: False-Negative Rapid Diagnostic Tests for Malaria and Deletion of the Histidine-Rich Repeat Region of the hrp2 Gene{dagger}. *Am J Trop Med Hyg* 2012, 86: 194-198.
8. Abdallah JF, Okoth SA, Fontecha GA, Torres RE, Banegas EI, Matute ML *et al*.: Prevalence of pfhrp2 and pfhrp3 gene deletions in Puerto Lempira, Honduras. *Malar J* 2015, 14: 19.
9. Amoah LE, Abankwa J, Oppong A: Plasmodium falciparum histidine rich protein-2 diversity and the implications for PfHRP 2: based malaria rapid diagnostic tests in Ghana. *Malar J* 2016, 15: 101.
10. Dorado EJ, Okoth SA, Montenegro LM, Diaz G, Barnwell JW, Udhayakumar V *et al*.: Genetic Characterisation of Plasmodium falciparum Isolates with Deletion of the pfhrp2 and/or pfhrp3 Genes in Colombia: The Amazon Region, a Challenge for Malaria Diagnosis and Control. *PLoS ONE* 2016, 11: e0163137.

11. Murillo SC, Akinyi OS, Abdallah JF, Pava Z, Dorado E, Incardona S *et al*.: Deletion of Plasmodium falciparum Histidine-Rich Protein 2 (pfhrp2) and Histidine-Rich Protein 3 (pfhrp3) Genes in Colombian Parasites. *PLoS ONE* 2015, 10: e0131576.
12. Li P, Xing H, Zhao Z, Yang Z, Cao Y, Li W *et al*.: Genetic diversity of Plasmodium falciparum histidine-rich protein 2 in the China-Myanmar border area. *Acta Trop* 2015, 152: 26-31.
13. Akinyi OS, Abdallah JF, Ceron N, Adhin MR, Chandrabose J, Krishnalall K *et al*.: Variation in Plasmodium falciparum Histidine-Rich Protein 2 (Pfhrp2) and Plasmodium falciparum Histidine-Rich Protein 3 (Pfhrp3) Gene Deletions in Guyana and Suriname. *PLoS ONE* 2015, 10: e0126805.
14. Wurtz N, Fall B, Bui K, Pascual A, Fall M, Camara C *et al*.: Pfhrp2 and pfhrp3 polymorphisms in Plasmodium falciparum isolates from Dakar, Senegal: impact on rapid malaria diagnostic tests. *Malar J* 2013, 12: 34.
15. Berhane A, Anderson K, Mihreteab S, Gresty K, Rogier E, Mohamed S *et al*.: Major Threat to Malaria Control Programs by Plasmodium falciparum Lacking Histidine-Rich Protein 2, Eritrea. *Emerg Infect Dis* 2018, 24: 462-470.
16. Beshir KB, Sepulveda N, Bharmal J, Robinson A, Mwanguzi J, Busula AO *et al*.: Plasmodium falciparum parasites with histidine-rich protein 2 (pfhrp2) and pfhrp3 gene deletions in two endemic regions of Kenya. *Sci Rep* 2017, 7: 14718.
17. Thomson R, Beshir KB, Cunningham J, Baiden F, Bharmal J, Bruxvoort KJ *et al*.: pfhrp2 and pfhrp3 Gene Deletions That Affect Malaria Rapid Diagnostic Tests for Plasmodium falciparum: Analysis of Archived Blood Samples From 3 African Countries. *J Infect Dis* 2019, 220: 1444-1452.
18. Thawer SG, Chacky F, Runge M, Reaves E, Mandike R, Lazaro S *et al*.: Sub-national stratification of malaria risk in mainland Tanzania: a simplified assembly of survey and routine data. *Malar J* 2020, 19: 177.
19. WHO. WHO Malaria Terminology. 2016. Geneva, Switzerland, World Health Organization.
20. Levy PS, Lemeshow S: *Sampling of populations: methods and applications*, 3rd edn. New York: John Wiley & Sons; 1999.
21. WHO. Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 8 (2016–2018). 2017. Geneva, Switzerland, World Health Organization.
22. Ranford-Cartwright LC, Taylor J, Umasunthar T, Taylor LH, Babiker HA, Lell B *et al*.: Molecular analysis of recrudescent parasites in a Plasmodium falciparum drug efficacy trial in Gabon. *Trans R Soc Trop Med Hyg* 1997, 91: 719-724.

9. Appendices

Appendix 1: Options for supplemental data collection

Appendix 2: Survey facility tally sheet

Appendix 3: Informed consent form (A – English and B – Kishwahili)

Appendix 4: Assent form template (A – English and B – Kishwahili)

Appendix 5: Survey report form (A – English and B – Kishwahili)

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

Appendix 7: List of HFs selected for the survey

Appendix 8: Curriculum vitae (CVs) of investigators

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 will be prioritized for analysis of *pfhrp2/3* gene deletions. This approach reduces the number of patient samples that need to be transported and analysed by PCR and 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. We have obtained sufficient human and financial resources to support analysis of a subset of DBS's from mRDT negative individuals for PCR and serology. This will enable us to determine the prevalence of *pfhrp2/3* gene deletions among PCR-confirmed low-density *P. falciparum* infections, missed by both mRDTs. In addition, DBS's from a subset or all malaria suspects positive for a *P. falciparum* infection by any diagnostic will be analysed, in order to confirm *pfhrp2/3* gene deletions among positive cases. This approach will identify infections caused by *pfhrp2* negative but *pfhrp3* positive parasites that still react with HRP2-based mRDTs due to cross-reactivity between HRP2 and HRP3, as well as multiclonal infections with parasites with and without *pfhrp2/3* deletion.

TABLE A1

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

HRP2 mRDT	pf-pLDH mRDT	Diagnosis	Order of priority for DBS analysis	Interpretation of results and limitations in detecting *pfhrp2/3* deletions
+	+	*P.falciparum*	2	• May be infection with *pfhrp2* deletion but HRP3 was detected by HRP2 mRDT • May be multiclonal infection with parasites with and without *pfhrp2/3* deletion
+	-	*P.falciparum*	3	• False-positive HRP2 mRDT (or persisting HRP2 after resolution of infection) • May be infection with *pfhrp2* deletion but HRP3 was detected by HRP2 mRDT • May be a low-density *P. falciparum* infection that does not result in pf-pLDH reaction with mRDT due to low antigen concentration • May be multiclonal infection with parasites with and without *pfhrp2/3* deletion
-	+	*P.falciparum*	1	• False-positive pf-pLDH mRDT • Low parasite density at limit of detection of mRDTs causing variable mRDT reactivity
-	-	Negative for malaria	4	• Cannot exclude low-density infection missed by both mRDTs, with undetected *pfhrp2/3* deletion. Use PCR to exclude malaria infection.

Appendix 2: *Pfhrp2/3* gene deletion survey facility tally sheet

This sheet will be filled out by all facilities. In each region, once 370 individuals with *P. falciparum* malaria have been seen (37 at each of the 10 enrolment facility per region), the proportion of discordant diagnoses (i.e. pf-pLDH positive AND HRP2 mRDT negative) among all positive *P.falciparum* diagnoses will be calculated by the regional supervisor and reported to the Project Manager. When centrally compiled (section 4.6 points 7 and 8), associated actions will be taken after statistical analysis of the molecular and serological confirmation data.

Table 2A: Tally sheet for collection of samples from health facilities

	A	B	C	D	E	F
	Suspected malaria case I.D.	**Date of visit / test (DD/MM/YYYY)**	**Informed consent**	**Pf case confirmed by at least one diagnostic method (Yes, No)**	**Cumulative number of Pf positive cases (from column D**	**Suspected false-neg HRP2 (pf-pLDH positive AND HRP2 mRDT negative)**
1			Y / N	Y / N		Y / N
2			Y / N	Y / N		Y / N
3			Y / N	Y / N		Y / N
4			Y / N	Y / N		Y / N
5			Y / N	Y / N		Y / N

Total malaria suspects tested (column A) _____

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

Number of suspected false-negative HRP2 mRDT results (column F – sum of 'yes' responses)

(a) _____

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

(b)

Percentage of all Pf cases with suspected false-negative HRP2 mRDT results that need molecular and serological analysis for *pfhrp2/3* deletions

(a / b) _____

Target: 37 *P. falciparum* cases detected per facility (last entry column F)

Appendix 3A: Informed consent form (English)
Survey ID: …… …….. *(**Insert a label here**)*

This informed consent form is for adults over age of 18 years who attend at …… Hospital/Health Centre/Dispensary (*name of health facility),* who have been invited to participate in a survey for *pfhrp2/3* deletions and biobanking for future malaria research

Name of principal investigator:	Dr. Deusdedith S. Ishengoma
Name of organization:	National Institute for Medical Research
Name of sponsor:	Bill and Melinda Gates Foundation
Name of proposal:	*Surveillance of pfhrp2/3 deletions and biobanking to support future research of malaria parasites in Mainland Tanzania*
Version:	HRP2/VERSION 1.0 of 30th June 2020

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 full informed consent form.

Part I: Information sheet

Introduction

I am …….. and I work with the National Institute for Medical Research (NIMR) under the Ministry of Health, Community Development, Gender, Elderly and Children. Today you (or your child) are invited to participate in a research study to better understand how well rapid diagnostic test kits for malaria are working in our 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 the Medical Research Coordinating Committee (MRCC) of NIMR, which is the Tanzanian Ethics 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 case of illiterate participants) in conjunction with the signature of an independent witness. A copy of this form will be provided to you and 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 have malaria and then use the blood samples to see if the malaria parasites have changed their form (characteristics) that can make them hard to be detected. We will ask you 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 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, district and region 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 a paper for further testing to see whether the malaria parasites have changes (mutations) making them hard to diagnose.

These tests will be carried out in Tanzania, the United States of America or any other country in the future based on expertise. 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 NIMR for 10-15 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 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, the principal investigator and project manager will be able to link your name to your survey identification number. The copy of 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 leftover 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) can also request that any leftover blood samples are withdrawn from long term storage.

Who do I contact if I have questions about the study?
If you have any questions you can contact the principal investigator,
Name**: Dr. Deusdedith Ishengoma** 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 communicate. You can also obtain more information about this survey by contacting the chairperson of MRCC,
Name: **Prof. Yunus D. Mgaya** Telephone number: **……**

Survey ID: …… ……..(*Insert a label here*)
Part II: Certificate of consent

Today's survey: I have read the consent form/it has been read and explained to me, and I agree to take part in the survey. I understand that I am free to choose for me or my child to be in this survey and that saying "NO" will have no effect on me/my child.		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___ / ___ / ___
Study staff	Name	Signature	Date___ / ___ / ___

***A participant or parent can sign or verbally state his/her consent and mark the form with a fingerprint in the presence of a witness who will then sign, in the event the subject cannot read the consent document. (Note: Date should be DD/MMM/YY)**

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

Your signature below means that you voluntarily agree to allow us to store mRDT and your filter paper blood sample for future studies:

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 mRDT/filter paper blood sample stored and used for future malaria 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___ / ___ / ____
Study staff	Name	Signature	Date___ / ___ / ____

***A participant or parent can sign or verbally state his/her consent and mark the form with a fingerprint in the presence of a witness who will then sign, in the event the subject cannot not read the consent document. (Note: Date should be in DD/MM/YY).**

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 for the samples to be stored for future use.

Print name of witness: _____ and thumbprint of participant:

Signature of witness: _____

Date: _____

dd/mmm/yyyy

Research Staff: I have accurately read or witnessed the accurate reading of the consent form to the participant who has had the opportunity to ask questions. I confirm that the individual has understood the information and given his/her consent freely.

Print name of the study staff: _____

Signature of the study staff _____

Date: _____ **(DD/MMM/YYYY)**

Appendix 3B: Fomu ya maombi ya ridhaa (Kiswahili)
Namba ya mshiriki (Survey ID): …… …….. *weka namba/lebo hapa (LABEL placed here)*

Fomu hii ya maombi ya ridhaa ni kwa ajili wa watu wazima wenye umri kuanzia miaka 18 na kuendelea ambao wamefika katika hospitali ya/kituo cha afya cha/zahanati ya ……., ambao wameombwa kushiriki katika utafiti wa kuangalia mabadiliko katika vimelea vya malaria yanayosababisha kipimo cha papo kwa papo cha malaria kisifanye kazi na kuanzisha mfumo wa kuhifadhi sampuli kwa ajili ya utafiti wa malaria katika siku za usoni (survey for *pfhrp2/3* deletions and biobanking).

Jina la Mtafiti Kiongozi:	Dr. Deusdedith S. Ishengoma
Jina la Taasisi:	Taasisi ya Taifa ya Utafiti wa Magonjwa ya Binadamu (NIMR)
Jina la Mfadhili:	Taasisi ya Bill na Melinda Gates (Bill and Melinda Gates Foundation)
Jina la utafiti:	*Utafiti wa utendaji wa kipimo cha malaria cha papo kwa papo na mfumo wa kuhifadhi sampuli kwa ajili ya utafiti wa malaria katika siku za usoni Tanzania Bara*
Toleo:	HRP2/Toleo la 1.0 la tarehe 30 Juni 2020

Fomu hii ya kuomba ridhaa ina sehemu mbili:

I. Maelezo kwa washiriki (kutoa taarifa kwa mshiriki kuhusu utafiti huu)
II. Uthibitisho wa ridhaa (kwa ajili ya kuweka sahihi kutoa idhini ya kushiriki kwenye utafiti)

Utapewa nakala ya fomu hii.

Sehemu ya I: Maelezo kwa washiriki
Utambulisho
Mimi ninaitwa ……, ninafanyakazi kwenye Taasisi ya Taifa ya Utafiti wa Magonjwa ya Binadamu (NIMR) iliyoko chini ya Wizara ya Afya, Maendeleo ya Jamii, Jinsia, Wazee na Watoto. Leo tunakualika wewe mwenyewe au mwanao kushiriki katika utafiti huu wenye

lengo la kuongeza ufahamu wetu wa jinsi kipimo cha malaria cha papo kwa papo kinachotumika hapa nchini kwetu kinavyofanya kazi. Tunakuomba ushiriki (au mwanao ashiriki) kwenye utafiti huu kwa sababu unazo dalili zinazoonesha kuwa huenda una ugonjwa wa malaria.

Andishi la utafiti huu limepitiwa na kuidhinishwa na kamati maalumu ya kuratibu tafiti za magonjwa ya binadamu (the Medical Research Coordinating Committee -MRCC-) iliyoko chini ya NIMR, na ambayo ndiyo bodi inayohusika na kudhibiti maadili ya utafiti wa afya nchini (the Tanzanian Ethics Review Board). Hakuna kazi yoyote ya utafiti itakayoanza kufanyika hadi utakapokuwa umepewa nafasi kupitia hii fomu, kuuliza maswali yoyote utakayokuwa nayo, na kisha kutoa ridhaa. Tunakuhimiza/kukushauri kuuliza maswali sasa na wakati wowote baadaye. Endapo utaamua kushiriki (au mwanao ashiriki), utaombwa kusaini fomu ya ridhaa au kuweka alama ya dole gumba (kama mshiriki hajui kusoma) na utachagua mwakilishi wako atakaye kuwa shahidi wako. Kama utaamua kushiriki, utapewa nakala ya fomu hii na ushiriki wako (au wa mwanao) ni wa hiari. Endapo utaamua kutoshiriki, uamuzi wako hautaathiri kwa namna yoyote huduma ya tiba ambayo utapewa na wahudumu wetu kwa ajili ya malaria au tatizo jingine,

Kwa nini utafiti huu unafanyika?
Tunafanya utafiti huu kwa sababu tunapenda kufanya uchambuzi wa sampuli za damu kutoka kwa wagonjwa wenye malaria ili kutafiti kama vimelea vya malaria vimejibadili au vimebadilika na kuvifanya visitambuliwe na kipimo cha malaria. Tutakuuliza maswali machache na kufanya vipimo zaidi ili kubainisha kama unaumwa (au mwanao anaumwa) malaria. Pia tutaomba idhini yako ili tutunze sampuli tukazochukua kutoka kwako kwa ajili ya tafiti za malaria katika siku za usoni.

Utafiti huu utafanyikaje? Nitatakiwa kufanya nini?
Endapo utakubali kushiriki kwenye utafiti huu, tutakuuliza maswali ya msingi kama vile umri wako, kijiji unachoishi, wilaya na mkoa wako, na vipimo ambavyo umefanyiwa au dawa ulizotumia hivi karibuni. Tutakapofanya kipimo cha papo kwa papo cha malaria, tutatumia sampuli hiyo ya damu kutoka kwenye ncha ya kidole kufanya kipimo kingine na tutaweka matone machache kwenye karatasi maalumu kwa ajili ya vipimo zaidi. Vipimo hivyo vitaonesha kama vimelea vya malaria vimebadilika kiasi kwamba haviwezi kugunduliwa kwa kutumia kipimo kinachotumika hivi sasa kupima malaria.
Vipimo vya ziada vitafanyika ndani na nje ya Tanzania, huko Marekani au nchi nyingine yoyote kutegemeana na mbinu mpya zitazogundulika katika siku za usoni. Kutakuwepo na mabaki ya sampuli baada ya kufanyika kwa vipimo vya kiuchunguzi. Badala ya kuharibu/kuteketeza sampuli hizi, tunaomba idhini

yako ili tuzihifadhi katika taasisi yetu ya NIMR kwa muda wa miaka 10 au 15. Baada ya hapo, sampuli hizo tutaziharibu/tutaziteketeza. Sampuli hizi zitatumika kwa ajili ya utafiti wa malaria na si vinginevyo; na tatifi hizo zitalenga kutafuta mbinu mpya za kufanya utambuzi wa vimelea vya malaria. Sampuli hizi hazitauzwa na matumizi yake yataidhinishwa/kusimamiwa na mamlaka za kitaifa zinazodhibiti/zinazosimamia tafiti za magonjwa ya binadamu.

Kuna madhara gani au usumbufu utakaotokana na kushiriki kwenye utafiti huu?

Kuna uwezekano mdogo sana wa kupata madhara kwa mtu yeyote atakayeshiriki kwenye utafiti huu. Pale utakapotobolewa kidole ili kuchukua sampuli, utapata maumivu kidogo na pia unaweza kupata mchubuko. Pia kuna unawezekano japo mdogo wa kupata maambukizi ya vimelea vya magonjwa mengine wakati tunachukua sampuli. Hata hivyo, tutatumia utaratibu ambao utafanyika kwa umakini ili kupunguza maumivu na kuondoa uwezekano wa kupata maambukizi au madhara mengine. Tunategemea kuwa, utapata usumbufu pale tutakapochukua muda wako wa dakika 10 au 15 ili kukamilisha dodoso la utafiti. Hata hivyo, utafiti huu utafanyika mara moja tu, na hautajirudia.

Pia kuna uwezekano japo mdogo kwamba mtu mwingine ambaye hahusiki na utafiti huu akaona taarifa zako za siri. Hata hivyo, jambo hili halitaweza kutokea kwa sababu hatutatumia jina lako (au jina la mwanao) kwenye dodoso la utafiti au fomu itakayoambatana na sampuli kwenda kwenye maabara zetu.

Kuna faida zozote kushiriki kwenye utafiti huu?

Faida za kushiriki katika utafiti huu ni kuwa leo tutakufanyia kipimo zaidi cha papo kwa papo cha malaria. Na endapo utatoa idhini tuchukue sampuli yako ya damu, ushiriki wako utaziwezesha mamlaka husika za afya ya jamiii kutoa huduma bora za vipimo vya malaria siku za usoni. Pia mamlaka husika zitaweza kutambua endapo vimelea vya malaria kutoka maeneo haya vinabadilika ili kukwepa kugundulika wakati wa kupima malaria.

Kuna gharama zozote zitakazotokana na ushiriki wako?

Ushiriki wako katika utafiti hauna gharama yoyote (ni bure), na pia hakuna malipo yoyote yatakayotolewa kwako endapo wewe mwenyewe utaamua (au mwanao ataamua) kushiriki kwenye utafiti huu.

Taarifa zangu binafsi zitalindwaje?

Tutachukua hatua na tahadhari zote kuhakikisha kuwa taarifa zako binafsi (au za mwanao) zinahifadhiwa vizuri na kwa siri. Kwa mfano, hatutatumia jina lako

(au la mwanao) au taarifa nyingine zinazoweza kukutambulisha kwenye dodoso la utafiti, sampuli ya damu au kwenye machapisho yetu. Badala yake, tutatumia namba maalumu ya utambulisho. Watafiti wanaohusika na zoezi hili la kuomba idhini yako, mtafiti kiongozi na meneja wa mradi ndio watakaoweza kuona na kuunganisha jina lako na nyaraka nyingine za utafiti zenye namba ya utambulisho. Nakala ya fomu ya maombi ya ridhaa ambayo itakuwa na jina lako na sahihi yako itahifadhiwa kwenye kabati maalumu (litakalofungwa wakati wote) na kwenye kompyuta ambazo zinatumia nywila (password) ili kulinda usiri wa taarifa zako. Watafiti watakaohusika na kuhifadhi sampuli yako (au ya mwanao) kwa ajili ya utafiti wa malaria katika siku za usoni hawatapewa jina lako.

Naweza kusitisha ushiriki wangu na haki zangu ni zipi?

Haulazimishwi kushiriki kwenye utafiti huu na hulazimiki kuturuhusu kutunza sampuli zako kwa ajili utafiti wa malaria katika siku za usoni. Endapo hutapenda kufanya hivyo, hutapata adhabu yoyote au kunyimwa huduma. Unaweza kubadili mawazo wakati wowote na kuondoa idhini yako (ya mwanao) kushiri kwenye utafiti huu bila madhara au adhabu yoyote kwako (au kwa mwanao). Unaweza kuwaagiza watifiti kuondoa sampuli yako kwenye mfumo wa kuhifadhi sampuli kwa ajili ya utafiti wa malaria katika siku za usoni.

Je, naweza kuwasiliana na nani endapo nina maswali kuhusu utafiti huu?

Endapo una maswali yoyote, unaweza kuwasiliana na Mtafiti kiongozi,
Jina: Dr. Deusdedith Ishengoma, kwa simu namba **……** .
Na endapo una maswali kuhusu haki zako au kama utapenda kuwasiliana na mtu ambaye siyo muhusika wa moja kwa moja wa utafiti huu, usisite kufanya hivyo. Unaweza kupata taarifa zaidi kuhusu utafiti huu kutoka kwa Mwenyekiti wa tume ya kuratibu tafiti za afya nchini (MRCC),
Jina: **Prof. Yunus D. Mgaya** Namba ya simu: **…….**

Nambari ya mshiriki (Survey ID): …… ……..(*Weka lebo/namba hapa)*

Sehemu ya II: Uthibitisho wa ridhaa

<table>
<tr><td colspan="3" rowspan="2"><u>Siku ya kwanza ya utafiti</u>: Nimesoma/nimesomewa na kupewa maelezo yaliyoko kwenye fomu ya maombi ya idhini, nakubali kushiriki kwenye utafiti huu. Natambua kuwa ninayo hiari ya kuamua mimi au mwanangu kuendelea kushiriki kwenye utafiti na endapo nitakataa sitapata au mwanangu hatapata madhara yoyote.</td><td colspan="2">Endapo unakubali, chagua NDIYO na kama hukubali chagua HAPANA.</td></tr>
<tr><td>NDIYO</td><td>HAPANA</td></tr>
<tr><td>Mshiriki mwenyewe au Mzazi/mlezi wa mtoto</td><td>Jina</td><td>Sahihi</td><td colspan="2">Tarehe___ / ___ / ___</td></tr>
<tr><td>Mtafiti</td><td>Jina</td><td>Sahihi</td><td colspan="2">Tarehe ___ / ___ / ___</td></tr>
</table>

***Mshiriki au Mzazi/mlezi anaweza kuweka sahihi au kutoa idhini kwa maneno na kisha kuweka dole gumba mbele ya shahidi, ambaye atasaini kwa niaba yake (endapo mshiriki hajui kusoma na kuandika). Tarehe iandikwe kwa kuonesha siku/Mwezi/Mwaka (DD/MMM/YYYY)**

Sahihi ya shahidi: Sahihi ya shahidi na alama ya dole gumba ya mshiriki vinahitajika endapo mshiriki hajui kusoma na kuandika. Katika hali hiyo, mshiriki atamchagua shahidi anayejua kusoma na kuandika ili asaini kwa niaba yake. Shahidi lazima achaguliwe na mshiriki mwenyewe na asiwe mmojawapo wa watafiti au asiwe na uhusiano wa moja kwa moja na watafiti. Nimeshuhudia kuwa mshiriki amesomewa fomu ya maombi ya ridhaa na amepewa nafasi ya kuuliza maswali. Nathibitisha kwamba mshiriki ametoa idhini ya kushiriki kwenye utafiti kwa hiari yake mwenyewe.

JIna la shahidi _____ Alama ya dole gumba la mshiriki:

Sahihi ya shahidi: _____

Tarehe: _____

dd/mmm/yyyy

Kwa kuwa umetia sahihi hapa chini, basi inaonesha kuwa umeamua kwa hiari yako kutoa idhini kwa watafiti kuhifadhi kipimo cha papo kwa papo kilichotumika na sampuli yako kwa ajili ya utafiti wa malaria katika siku za usoni.

Kwa ajili ya kuhifadhi sampuli kwa matumizi ya utafiti siku za usoni: Kwa hiari yangu natoa idhini kwa watafiti kuhifadhi sampuli yangu (au ya mwanangu) kwa matumizi ya utafiti wa malaria kwa siku za usoni. Naelewa kuwa naweza kubadili nia yangu na kuomba sampuli yangu iondolewe kwenye mfumo huo wa kuhifadhi sampuli kwa ajili ya utafiti wa malaria kwa siku za usoni.	Endapo unakubali chagua NDIYO na kama hukubali chagua HAPANA.	
	NDIYO	HAPANA

Mshiriki au Mzazi/mlezi	Jina	Sahihi	Tarehe:___ / ___ / ____
Mtafiti	Jina	Sahihi	Tarehe: ___ / ___ / ____

***Mshiriki au Mzazi/mlezi anaweza kuweka sahihi au kutoa idhini kwa maneno na kisha kuweka dole gumba mbele ya shahidi, ambaye atasaini kwa niaba yake (endapo mshiriki hajui kusoma na kuandika). Tarehe iandikwe kwa kuonesha siku/Mwezi/Mwaka; (DD/MMM/YYYY)**

Sahihi ya shahidi: Sahihi ya shahidi na alama ya dole gumba ya mshiriki vinahitajika endapo mshiriki hajui kusoma na kuandika. Katika hali hiyo, mshiriki atamchagua shahidi anayejua kusoma na kuandika ili asaini kwa niaba yake. Shahidi lazima achaguliwe na mshiriki mwenyewe na asiwe mmojawapo wa watafiti au asiwe na uhusiano wa moja kwa moja.

Nimeshuhudia kuwa mshiriki amesomewa fomu ya maombi ya ridhaa na amepewa nafasi ya kuuliza maswali. Nathibitisha kwamba mshiriki ametoa idhini ya kushiriki kwenye utafiti kwa hiari yake mwenyewe.

JIna la shahidi _____ Alama ya dole gumba la mshiriki:

Sahihi ya shahidi: _____

Tarehe: _____

dd/mmm/yyyy



Appendix 4A: Assent form (English) for minors ages 7–<17

Survey ID**: …… ……..(*INSERT A LABEL HERE*)**

Name of principal investigator:	Dr. Deusdedith S. Ishengoma
Name of organization:	National Institute for Medical Research
Name of sponsor:	Bill and Melinda Gates Foundation
Name of proposal:	Surveillance of *pfhrp2/3* deletions and biobanking to support future research of malaria parasites in Mainland Tanzania
Version:	HRP2/VERSION 1.0 of 30th June 2020

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 full informed consent form.

Part I: Information sheet

Introduction

I am …….. and I work with the National Institute for Medical Research (NIMR) under the Ministry of Health, Community Development, Gender, Elderly and Children. Today you are invited to participate in a research study to better understand how well rapid diagnostic test kits for malaria are working in our country. You 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 the Medical Research Coordinating Committee (MRCC) of NIMR, which is the Tanzanian Ethics Review Board. No research activity will be conducted until you have had an opportunity to review this assent form, ask any questions you may have, and provide an assent. We encourage you to ask questions now and at any time. If you decide to participate, you will be asked to sign the assent form. A copy of this form will

be provided to you and your participation is completely voluntary and will in no way affect the treatment and care you receive for malaria or any other condition.

There may be some leftover blood after we conclude the routine testing and survey today and instead of discarding it, we would like your permission to store any such leftover blood for 10-15 years, afterwards it will be destroyed. This survey is done to better understand how well rapid diagnostic test kits work in our country and to help make better tests in the future. Please keep in mind that your parent has already agreed to participation, but you are also free to decide for yourself.

Why are we creating this resource of leftover blood samples (biobank)

We are creating this collection of left over blood samples to see if the parasite is changing and finding ways to escape detection of the current malaria rapid diagnostic tests, this means give a negative result when they shouldn't. We want to let scientists use these samples in their research to make new tests for malaria that are not affected by these changes in the parasite.

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

If you agree to take part, there are no additional procedures

What are the risks or inconveniences of the study?

There is very little risk of harm to anyone who agrees to participate.

What are the benefits of the study?

There will be no direct benefit to you for taking part in this study.

Are there costs to participate?

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

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

You do not have to allow us to store your leftover blood samples if you do not want to. You are free to choose if you want to. You are free to talk to your parents or the research team.

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

If you have any questions about this research you can contact the principal investigator, Name: **Dr. Deusdedith Ishengoma** and Telephone number: **……**

If you have any questions about your rights, or if you want to talk with someone who is not part of this research project, please make communication. You can also obtain my information about this survey by contacting the chairperson of MRCC,
Name: **Prof. Yunus D. Mgaya** Telephone number: **……**

Survey ID: ……(*Insert a Label here*)

Statement of permission

By signing or placing my thumbprint below, I agree that:

- I have read this form or it has been read to me;
- I have been able to ask questions about it. The question were answered to my satisfaction;
- I choose to be in this evaluation;
- I have been told that I can drop out from this evaluation at any time.

Child Assenting	Name	Signature/print	Date___ / ___ / ___

-

*** I have witnessed the informed permission and can attest that the child has been given details of the evaluation, told about the risks and benefits, and had the opportunity to have questions answered. (Note: Date should be DD/MMM/YYYY)**

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

Print name of the study staff:_____

Signature of the study staff: _____

Date:___ / ___ / ___

Appendix 4B: Fomu ya kuomba ridhaa ya kushiriki kwenye utafiti kwa watoto wenye umri wa kuanzia miaka 7 hadi 18.

Namba ya mshiriki (Survey ID): *…… …….. weka namba/lebo hapa (LABEL placed here)*

Jina la Mtafiti Kiongozi:	Dr. Deusdedith S. Ishengoma
Jina la Taasisi:	Taasisi ya Taifa ya Utafiti wa Magonjwa ya Binadamu (NIMR)
Jina la Mfadhili:	Taasisi ya Bill na Melinda Gates (Bill and Melinda Gates Foundation)
Jina la utafiti:	*Utafiti wa utendaji wa kipimo cha malaria cha papo kwa papo na mfumo wa kuhifadhi sampuli kwa ajili ya utafiti wa malaria katika siku za usoni Tanzania Bara*
Toleo:	HRP2/Toleo la 1.0 la tarehe 30 Juni 2020

Fomu hii ya kuomba ridhaa ina sehemu mbili:

I. Maelezo kwa washiriki (kutoa taarifa kwa mshiriki kuhusu utafiti huu)
II. Uthibitisho wa ridhaa (kwa ajili ya kuweka sahihi kutoa idhini ya kushiriki kwenye utafiti)

Utapewa nakala ya fomu hii.

Sehemu ya I: Maelezo kwa washiriki

Utambulisho

Mimi ninaitwa ……, ninafanyakazi kwenye Taasisi ya Taifa ya Utafiti wa Magonjwa ya Binadamu (NIMR) iliyoko chini ya Wizara ya Afya, Maendeleo ya Jamii, Jinsia, Wazee na Watoto. Leo tunakualika kushiriki katika utafiti huu wenye lengo la kuongeza ufahamu wetu wa jinsi kipimo cha malaria cha papo kwa papo kinachotumika hapa nchini

kwetu kinavyofanya kazi. Tunakuomba ushiriki kwenye utafiti huu kwa sababu unazo dalili zinazoonesha kuwa huenda una ugonjwa wa malaria.
Andishi la utafiti huu limepitiwa na kuidhinishwa na kamati maalumu ya kuratibu tafiti za magonjwa ya binadamu (the Medical Research Coordinating Committee -MRCC-) iliyoko chini ya NIMR, na ambayo ndiyo bodi inayohusika na kudhibiti maadili ya utafiti wa afya nchini (the Tanzanian Ethics Review Board). Hakuna kazi yoyote ya utafiti itakayoanza kufanyika hadi utakapokuwa umepewa nafasi kupitia hii fomu, kuuliza maswali yoyote utakayokuwa nayo, na kisha kutoa ridhaa. Tunakuhimiza/kukushauri kuuliza maswali sasa na wakati wowote baadaye. Endapo utaamua kushiriki, utaombwa kusaini fomu ya ridhaa au kuweka alama ya dole gumba (kama mshiriki hajui kusoma) na utachagua mwakilishi wako atakaye kuwa shahidi wako. Kama utaamua kushiriki, utapewa nakala ya fomu hii na ushiriki wako ni wa hiari. Endapo utaamua kutoshiriki, uamuzi wako hautaathiri kwa namna yoyote huduma ya tiba ambayo utapewa na wahudumu wetu kwa ajili ya malaria au tatizo jingine. Kuna uwezekano kuwa kuna sampuli za damu ambazo zitabaki baada ya kukamilisha shughuli za utafiti leo. Badala ya kuharibu/kuteketeza sampuli hizo, tunaomba idhini yako ili tuhifadhi sampuli hizo kwa miaka kati ya 10 na 15; baada ya hapo tutaziharibu/tutaziteketeza. Utafiti huu unafanyika ili tuweze kupata taarifa na uelewa mzuri wa jinsi kipimo cha papo kwa papo cha malaria kinavyofanyakazi hapa nchini mwetu na utasaidi katika utegenezaji wa vipimo bora vya malaria katika siku za usoni. Naomba utambue kuwa mzazi/mlezi wako tayari ameshatoa idhini ya kushiriki kwenye utafiti huu. Hata hivyo, tungependa na wewe mwenyewe utoe uamuzi wako kama utapenda kushiriki kwenye huu utafiti huu au la.

Kwa nini tunaanzisha mfumo wa kuhifadhi sampuli za damu kwa ajili ya utafiti wa malaria (biobank)?

Tunaanzisha mfumo wa kukusanya na kuhifadhi sampuli za damu kwa ajili ya utafiti wa malaria ili kufanya tafiti kwa ajili ya kuona kama vimelea vya malaria vimebadilika ili kukwepa kugundulika na kipimo cha malaria kinachotumika sasa. Hii inamaanisha kuwa kipimo cha malaria kinaweza kutoa majibu kuwa hakuna malaria wakati mgonjwa ana vimelea vya malaria katika damu yake. Tungependa pia kutoa fursa kwa watafiti wetu kutumia sampuli tunazokusanya ili kufanya utafiti kwa ajili ya kugundua vipimo vipya vya malaria ambavyo vitatatua tatizo linalotokana na mabadiliko ya vimelea.

Utafiti huu utafanyikaje? Nitatakiwa kufanya nini?

Endapo utakubali kushiriki kwenye utafiti huu, hakutakuwa na kitu kingine kitakachofanyika zaidi ya yale ambayo tayari mzazi wako ameelezwa.

Kuna madhara gani au usumbufu utakaotokana na kushiriki kwenye utafiti huu?
Kuna uwezekano mdogo sana wa kupata madhara kwa mtu yeyote atakayeshiriki kwenye utafiti huu.

Kuna faida zozote kushiriki kwenye utafiti huu?
Hakuna faida ya moja kwa moja ambayo utaipata kwa kushiriki katika utafiti.

Kuna gharama zozote zitakazotokana na ushiriki wangu?
Ushiriki wako katika utafiti hauna gharama yoyote (ni bure), na pia hakuna malipo yoyote yatakayotolewa kwako endapo utaamua kushiriki kwenye utafiti huu.

Naweza kusitisha ushiriki wangu na haki zangu ni zipi?
Haulazimishwi kuturuhusu kuhifadhi sampuli zako kwa ajili utafiti wa malaria katika siku za usoni kama hutapenda kufanya hivyo. Unao uhuru wa kuamua kushiriki au la. Pia uko huru kuongea na wazizi/walezi wako au mmojawapo wa watafiti kuhusu ushiriki wako.

Je, naweza kuwasiliana na nani endapo nina maswali kuhusu utafiti huu?
Endapo una maswali yoyote, unaweza kuwasiliana na Mtafiti kiongozi,
Jina: Dr. Deusdedith Ishengoma, kwa simu namba **……** .
Na endapo una maswali kuhusu haki zako au kama utapenda kuwasiliana na mtu ambaye siyo muhusika wa moja kwa moja wa utafiti huu, usisite kufanya hivyo. Unaweza kupata taarifa zaidi kuhusu utafiti huu kutoka kwa Mwenyekiti wa tume ya kuratibu tafiti za afya nchini (MRCC),
Jina: **Prof. Yunus D. Mgaya** Namba ya simu: **…….**

Nambari ya mshiriki (Survey ID): ……..(*Weka lebo/namba ya mshiriki hapa)*

Sehemu ya II: Ridhaa ya kushiriki kwenye utafiti

Kwa kuwa nimeweka sahihi au alama dole gumba hapa chini, nimekubali kwamba

- Nimesoma fomu ya maelezo kwa washiriki wa utafiti huu au nimesomewa formu hiyo
- Nimepewa nafasi na nimeweza kuuliza maswali
- Nimeamua kwa hiari yangu kushiriki kwenye utafiti huu
- Nimehakikishiwa kuwa naweza kubadilisha mawazo na kujitoa kwenye utafiti huu wakati wowote.

Mtoto anayetoa idhini	Jina	Sahihi/dole gumba	Tarehe: ___ / ___ / ___

-

***Nimeshuhudia mhusika akitoa idhini ya kushiriki baada ya kupewa taarifa za utafiti na ninathibitisha kuwa mtoto huyu amepewa taarifa za kina za utafiti, ameelezwa madhara na faida za kushiriki kwenye utafiti, na pia amepewa fursa ya kuuliza maswali ambayo yamejibiwa na watafiti. Tarehe itaandikwa kwa kuanza na siku, mwezi na mwaka (DD/MMM/YYYY)**

Nimemsomea mtoto au amesomewa fomu ya kutoa idhini ili kushiriki kwenye utafiti, na mshiriki amepewa fursa ya kuuliza maswali. Nathibitisha kuwa mshiriki ametoa idhini ya kushiriki kwa hiari yake mwenyewe.

Jina la mtafiti: _____

Sahihi ya mtafiti:_____

Tarehe: ___ / ___ / ___

Appendix 5A: Survey case report form surveillance + biobanking **(English)**

The survey form will be pre-labelled chronologically and similar labels will be placed on mRDTs, DBSs and plastic bags. The *form will be produced in duplicate –. One copy will stay with the regional supervisor for analysis of indicator 1, and the other will go to the lab with filter paper for analysis of indicators 2 and 3.*

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

To be completed for all suspected malaria patients		
1	Patient ID (place a label)	
2	Name of Health facility	
3	Name of health worker/ laboratory Personnel	
4	Date of visit (DD/MMM/YYYY)	Day____ Month_____ Year_____

5	mRDT 1 (HRP2/PAN) Product Code: Lot number: Expiry date:	 **Box 1** Control Pan **P.f** **HRP2** + / - + / - + / - *Circle correct result in each box above.* Circle result of RDT: 1. Negative 2. *P.falciparum* *3. Pan*

6	mRDT 2 (Survey mRDT) SD Malaria Ag P.f (HRP2/pLDH) (05FK90) Product Code: Lot number: Expiry date:	 Box 2 Control T2 Pf-LDH T1 HRP2 + / - + / - + / - *Circle correct result in each box above.* Circle result of RDT: 1. Negative *2. P. falciparum*
7	a. Is mRDT1 positive for *P. falciparum or non-falciparum malaria* ? b. Is mRDT2 positive for *P. falciparum*?	Y / N Y / N If YES to EITHER question, provide treatment.
8	DBS taken?	Y / N

Questions for all participant:		
9	Date of birth (DD/MMM/YYYY) or Age (***years***)	Day____ Month_____ Year_____ or age_____years
10	Sex (***circle appropriate number***)	1. Male 2. Female _____
11	Residence (village/street, district, region)	Village/street _____ District _____ Region _____
12	In the past 2 weeks, have you had a test for malaria? (***circle correct number***)	1. No Go to question 15 2. Yes
13	What was the result of the test? (***circle correct number)***	1. Positive 2. Negative 3. Don't know
14	In the past 2 weeks, have you taken any medicine for malaria? (***circle correct number)***	1. No Go to question 17 2. Yes
15	Antimalarial medicine taken (***circle correct number)***	*(multiple answers allowed)* 1. Artemether-lumefantrine / Coartem 2. Others, specify _____
16	Have you travelled and spent over night to another locality or country for the past 30 days? (***circle correct number)***	1. No end 2. Yes go to question 17
17	Where did you travel?	

A	First area	a. Country_____ b. Region _____ c. District_____ d. Village_____
	How many days did you spend there?	_____ days
b.	Second area	a. Country_____ b. Region _____ c. District_____ d. Village_____
	How many days did you spend there?	_____ days
c.	Third area	a. Country_____ b. Region _____ c. District_____ d. Village_____
	How many days did you spend there?	_____ days

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		
L1.	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
L2.	Serology	a. pfhrp2+/pan-LDH+ b. pfhrp2-/pan-LDH- c.pfhrp2+/pan-LDH- d.pfhrp2-/pan-LDH+ e. other antigens (specify)_____


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

	Suspected false-negative HRP2 mRDT prevalence[b] (n=XX)	*Confirmed pfhrp2/3* deletion prevalence[c] (n=XX)
Characteristic	**(95% CI)**	**(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		
Region (survey domain)		
Region 1		
Region 2		
Region 3		
Region 4		
Region 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.

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

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


Appendix 7: List of selected health facilities

	HF Name	Type	District	Region		HF Name	Type	District	Region
	High transmission regions					Moderate transmission			
1	Mkutimango	Dispensary	Mtwara DC	Mtwara	1	Izengabatogil we	Dispensary	Urambo	Tabora
2	Shangani	Dispensary	Mtwara TC		2	Migungumalo	Dispensary	Uyui	
3	Lumesule	Dispensary	Nanyumbu		3	Nzigala	Dispensary	Uyui	
4	Maratani	Dispensary	Nanyumbu		4	Semembela	Dispensary	Nzega DC	
5	Sengenya	Dispensary	Nanyumbu		5	Uhemeli	Dispensary	Nzega DC	
6	Chiwonga	Dispensary	Newala DC		6	Usunga	Dispensary	Sikonge	
7	Mpalu	Dispensary	Newala DC		7	Utuja	Dispensary	Igunga	
8	Nanyamba	Dispensary	Newala DC		8	Izimbili	Dispensary	Urambo	
9	Michenjele	Dispensary	Tandahimb a		9	Kasimana	Dispensary	Kaliua	
10	Mkunya	Health Centre	Newala TC		10	Kitunda	Health Centre	Sikonge	
1	Kasozibaka	Dispensary	Biharamulo	Kagera	1	Kisorya	Health Centre	Bunda	Mara
2	Bukangara	Dispensary	Karagwe		2	Raranya	Dispensary	Rorya	
3	Rwambere	Dispensary	Kyerwa		3	Panyakoo	Dispensary	Rorya	
4	Rugasha	Dispensary	Kyerwa		4	Kenyamanyori	Dispensary	Tarime	
5	Rushwa	Dispensary	Muleba		5	Nyagere	Dispensary	Bunda	
6	Buyongo	Dispensary	Misenyi		6	Nyamburundu	Dispensary	Bunda	
7	Nterugwe	Dispensary	Ngara		7	Marambeka	Dispensary	Bunda	
8	Chivu	Dispensary	Ngara		8	Chitare disp	Dispensary	Musoma DC	
9	Kakolaijo	Dispensary	Karagwe		9	Chereche	Dispensary	Rorya	
10	Nshamba	Health Centre	Muleba		10	Kibuyi	Dispensary	Rorya	
	Low transmission regions					Very low transmission regions			
1	Tunduma	Health Centre	Tunduma	Songwe	1	Kagongo	Health Centre	Mwanga	Kilimanjaro
2	Kapeta	Dispensary	Illeje		2	KIA	Dispensary	HAI	
3	Ileya	Dispensary	Songwe Dc		3	Kirungu	Dispensary	Moshi DC	
4	Ngulugulu	Dispensary	Illeje		4	Mwangaria	Dispensary	Moshi DC	
5	Udinde	Dispensary	Songwe Dc		5	Karamba	Dispensary	Mwanga	
6	Itentula	Dispensary	Mbozi DC		6	Kalemawe	Dispensary	Same	
7	Utambalile	Dispensary	Mbozi DC		7	Manyata	Dispensary	Siha	

8	Chilulumo	Dispensary	Momba DC		8	Sanya juu	Dispensary	Siha	
9	Ivuna	Dispensary	Momba DC		9	Rongai	Dispensary	Rombo	
10	Chiwezi	Dispensary	Tunduma		10	Mengwe Juu	Dispensary	Rombo	
1	Mauno	Health Centre	Kondoa DC	Dodoma	1	Nkomang'ombe	Dispensary	Ludewa	Njombe
2	Chogola	Dispensary	Mpwapwa		2	Ikondo	Dispensary	Njombe DC	
3	Mindola	Dispensary	Bahi		3	Mbugani	Dispensary	Makambako	
4	Ikombolinga	Dispensary	Chamwino		4	Madeka	Dispensary	Njombe DC	
5	Manda	Dispensary	Chamwino		5	Idundilanga	Dispensary	Njombe Mc	
6	Babayu	Dispensary	Chemba		6	Igwachanya	Dispensary	Wang'ing'ombe	
7	Kinyamshindo	Dispensary	Chemba		7	Palagavanu	Dispensary	Wang'ing'ombe	
8	Mbabala A	Dispensary	Dodoma MC		8	Ikuna	Dispensary	Njombe Dc	
9	Kwadelo	Dispensary	Kondoa DC		9	Kifumbe	Dispensary	Makambako	
10	Kibaigwa	Dispensary	Kongwa		10	Manda HC	Health Centre	Ludewa	
1	Chanika	Health Centre	Ilala	Dar	1	Kibaya	Hospital	Kiteto DC	Manyara
2	Mbezi	Health Centre	Ubungo		2	Galapo	Health Centre	Babati DC	
3	Vingunguti	Dispensary	ilala		3	Kateshi	Health Centre	Hanang DC	
4	Kibada	Dispensary	Kigamboni		4	Kiru	Dispensary	Babati DC	
5	Pembamnazi	Dispensary	Kigamboni		5	Sabilo	Dispensary	Babati TC	
6	Kawe	Dispensary	Kinondoni		6	Dirma	Dispensary	Hanang	
7	Mwenge	Dispensary	Kinondoni		7	Lengatei	Dispensary	Kiteto	
8	Chamazi	Dispensary	Temeke		8	Yaeda Chini	Dispensary	Mbulu DC	
9	Mzinga	Dispensary	Temeke		9	Loiborsoit B	Dispensary	Simanjiro	
10	Goba	Dispensary	Ubungo		10	Terrat	Dispensary	Simanjiro	

Appendix 8: Curriculum vitae (CVs) of investigators (to be attached)