A Malaria Elimination Guide to Targeted Surveillance and Response in High-Risk Populations

Module 2: Identifying Risk Factors Using Case-Control Studies

The Malaria Elimination Initiative

UCSF Institute for Global Health Sciences

Contents

Acronyms and Key Terms

Active case detection (ACD)	Detection by health workers of malaria cases at community and household levels, sometimes in population groups that are considered at high risk. Active case detection can consist of screening for fever followed by parasitological examination of all febrile patients or as parasitological ex amination of the target population without prior screening for fever.
Case, imported	Malaria case or infection in which the infection was acquired outside the area in which it is diagnosed. In keeping with the WHO Surveillance oper ations manual, the origin of imported cases can be traced to a known ma larious area outside of the elimination area to which the case has travelled.
Case, index	A case of which the epidemiological characteristics trigger additional active case or infection detection. The term "index case" is also used to designate the case identified as the origin of infection of one or a number of introduced cases.
Case, locally acquired	A case acquired locally by mosquito-borne transmission.
Confirmed malaria case	Malaria case (or infection) in which the parasite has been detected in a diagnostic test, i.e. microscopy, a rapid diagnostic test or a molecular diagnostic test.
Case notification	Compulsory reporting of all malaria cases by medical units an medical practitioners to either the health department or the malaria control program, as prescribed by national laws or regulations
Control[1]	A subject who does not have the outcome of interest and is therefore a member of a comparison group to which those with the outcome (the 'cases') are compared. In this case, a control is a person who tests negative for malaria.
Dried Blood Spots (DBS)	A method of collecting and storing blood samples on a filter paper with stamped rings on which finger-prick blood is collected. Dried blood spots are a source of DNA for PCR-based detection and genotyping of malaria parasites in remote field settings with limited laboratory facilities and where cold chain storage is not available.
Exposure[1]	A term used to indicate that a study subject has a particular risk factor. For example, exposure to cross-border travel was defined as traveling internationally within the past 30 days.
Loop-mediated isothermal amplification (LAMP)	A molecular detection technique that has very high sensitivity and specificity to detect very low levels of asymptomatic infections in both high and low transmission settings. It amplifies nucleic acid under isothermal conditions and is simpler and faster to use than PCR.
Outcome[1]	A general term for the endpoint of a study, such as occurrence of a disease. In this case, the outcome is malaria status.

Definitions were reproduced from WHO malaria terminology unless referenced otherwise.

WHO malaria terminology. (2017, June). Retrieved July 27, 2017, from http://apps.who.int/iris/bitstream/10665/208815/1/WHO_HTM_GMP_2016.6_eng.pdf

1   Hulley, S. B., Cummings, S. R., & Browner, W. S. (2013). Designing clinical research (4th ed.). Philadelphia , PA: Wolters Kluwer Health Lippincott Williams & Wilkins.

Passive case detection (PCD)	Detection of malaria cases among patients who, on their own initiative, visit health services for diagnosis and treatment, usually for a febrile illness.
Polymerase chain reaction (PCR)	A technique used in molecular biology to "amplify" small segments of DNA or a gene
Reactive case detection (RACD)	Screening and testing provided to a subset of a population in a given area in response to the detection of an infected person. Typically carried out around the index case household within a given radius
Source population[1]	Population that would have been included in a cohort study (if cases were to arise prospectively) and which gives rise to the cases.

Overview of Module 2

Module 2 provides instructions on how to conduct an assessment of malaria risk factors using a standard case-control design. This design, termed the Malaria Elimination Risk Factor Assessment Tool (MERFAT), is useful across a wide range of settings and operationally simple as it uses cases and controls identified from health facilities.

This Module provides detailed documentation, standard operating procedures (SOPs), and data collection tools to enable project staff to identify and quantify actionable risk factors for malaria high-risk populations (HRPs). This information can be used to identify how and where infections were acquired, thus target interventions to specific locations and population sub-groups.
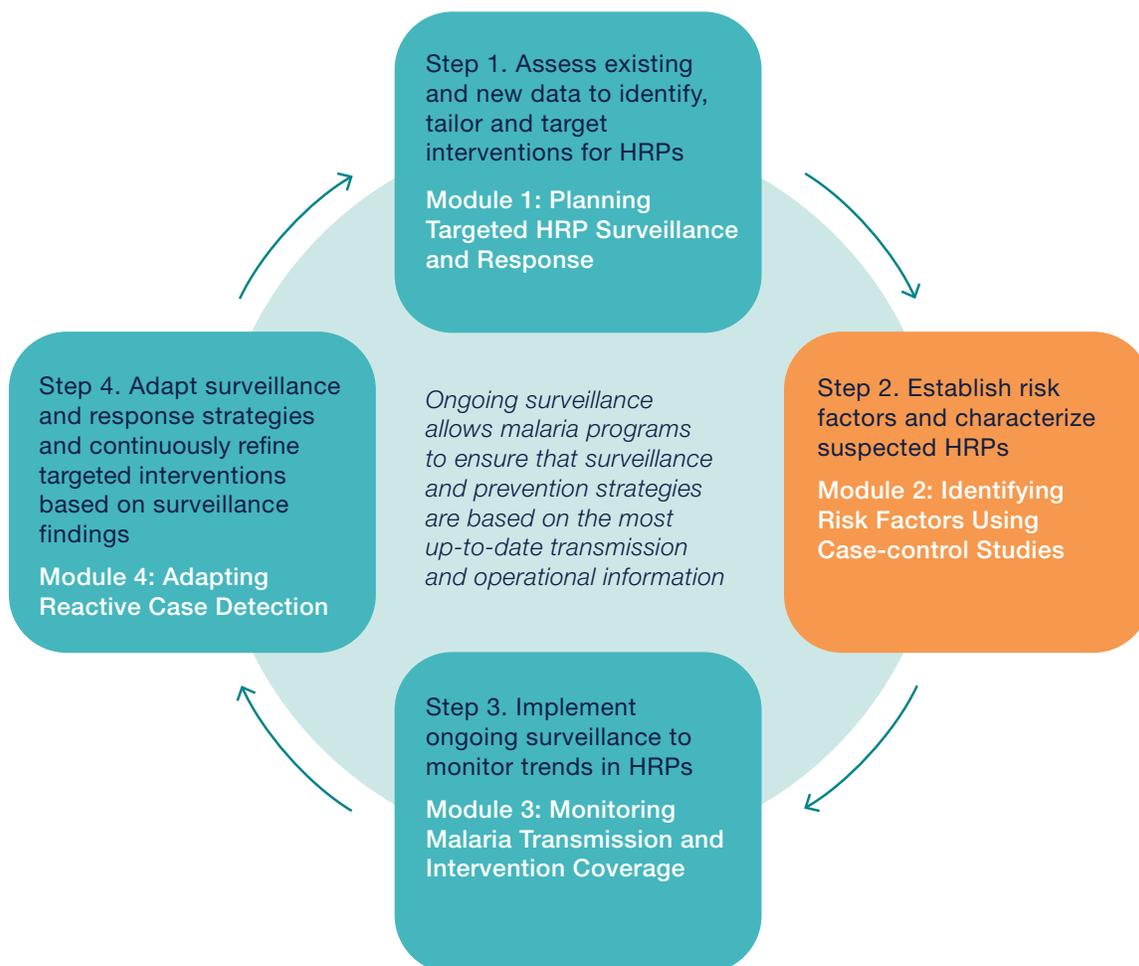
Module 2 links with the other modules in the HRP Guide (Figure 1).

The Module is organized into three components:

1. Planning the MERFAT, including funding, forming the study team and adapting the design and questionnaire.

2. Data collection which entails detailing staff roles, preparation of materials, implementing data collection and managing documentation and data.

3. Data analysis

The data collection section provides detailed SOPs that can be reviewed during training and adapted to suit the country specific context. MERFAT data collection tools are provided in the appendices and in the links within this Module.

Figure 1: Generating and using evidence: steps in the surveillance cycle for targeting HRPs

Step 1. Assess existing and new data to identify, tailor and target interventions for HRPs

Module 1: Planning Targeted HRP Surveillance and Response

Step 2. Establish risk factors and characterize suspected HRPs

Module 2: Identifying Risk Factors Using Case-control Studies

Step 3. Implement ongoing surveillance to monitor trends in HRPs

Module 3: Monitoring Malaria Transmission and Intervention Coverage

Step 4. Adapt surveillance and response strategies and continuously refine targeted interventions based on surveillance findings

Module 4: Adapting Reactive Case Detection

Ongoing surveillance allows malaria programs to ensure that surveillance and prevention strategies are based on the most up-to-date transmission and operational information

Introduction to MERFAT

What is MERFAT?

The Malaria Elimination Risk Factor Assessment Tool (MERFAT) was developed to help programs and partners identify or confirm malaria HRPs and underlying risk factors for infection. MERFAT uses a case-control methodology to compare people with malaria (cases) to those without malaria (controls).

The tool consists of guidelines, sample protocols and questionnaires to help programs and partners design, implement and interpret the findings of a standard assessment. Results can be used to support decision-making around resource allocation for targeted surveys and/or intervention strategies, as well as tailor intervention packages for a specific group.

When, Where and Why Use MERFAT?

MERFAT can provide evidence of who is at highest risk of malaria in a given locality and where gaps in intervention coverage lie.

MERFAT case-control methodology is particularly suited to low-endemic and elimination settings, where there are generally few malaria cases. In these contexts, population-based surveys (e.g., malaria indicator surveys) are unlikely to detect enough cases to provide programmatically useful information about HRPs. Figure 2 presents a decision tree on when and where MERFAT can be applied.
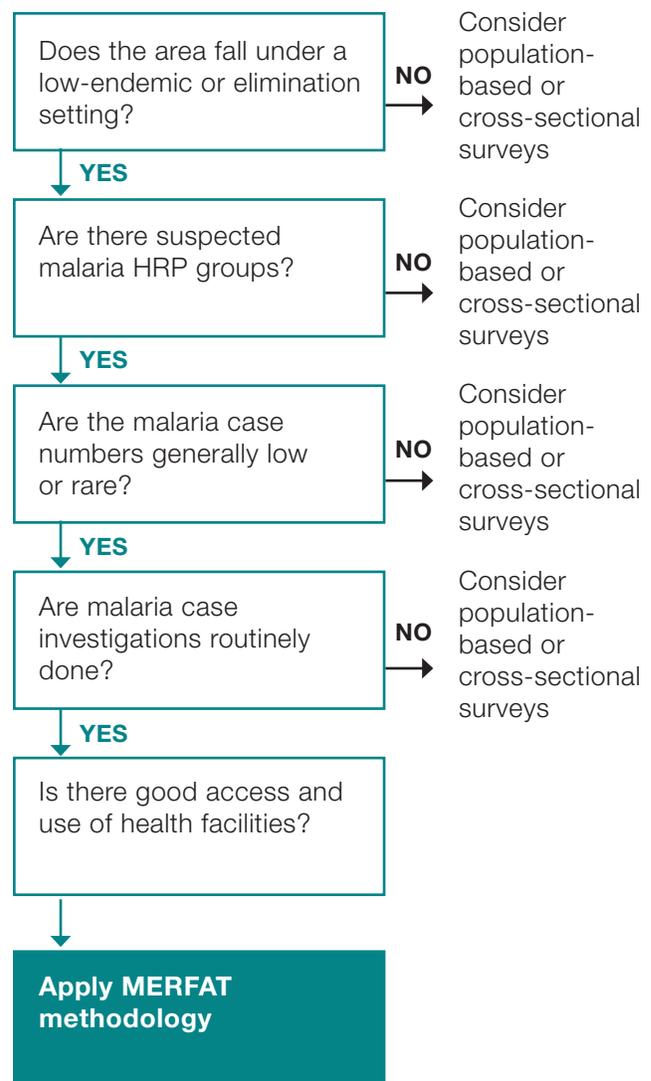
In contexts where case investigations are routinely carried out at the point of care, and where there is good access to health services, MERFAT can easily be integrated into existing surveillance systems by administering a set of core questions in the case investigation form to a control group in order to provide a suitable comparison.

The MERFAT approach has been used to identify specific malaria risk factors in Indonesia (Box 1) and other countries.

Who Should Use MERFAT?

MERFAT was developed for use by health decision makers, malaria program managers, nongovernmental organizations, and researchers who want to generate evidence to improve surveillance and support targeted intervention strategies for populations at increased risk of malaria.

Figure 2: MERFAT Decision Tree

Does the area fall under a low-endemic or elimination setting? — **NO** → Consider population-based or cross-sectional surveys

YES ↓

Are there suspected malaria HRP groups? — **NO** → Consider population-based or cross-sectional surveys

YES ↓

Are the malaria case numbers generally low or rare? — **NO** → Consider population-based or cross-sectional surveys

YES ↓

Are malaria case investigations routinely done? — **NO** → Consider population-based or cross-sectional surveys

YES ↓

Is there good access and use of health facilities?

↓

Apply MERFAT methodology

Box 1. MERFAT in Indonesia

Although Aceh Province, Indonesia, achieved a decline in malaria incidence from 0.9 to 0.1 cases per 1,000 population between 2010 and 2015, locally acquired cases have continued to occur past the province's goal of elimination by 2015. Preliminary studies conducted by the UCSF Malaria Elimination Initiative have found that malaria in Aceh Province clusters socio-demographically and often among individuals who work or sleep in the forest. In order to better define populations at high risk of malaria and gaps in intervention coverage in Aceh Province, MERFAT was conducted using passively detected cases and controls from 2017–2018 in five subdistricts. This study provided the opportunity to explore risk factors in the categories of occupation, socioeconomic, housing, and non-forest and forest-related behaviors. Preliminary findings show that 72.3% of cases, 18.8% of health facility controls, and 8.7% of community controls reported one trip to the forest.

Information gleaned from this type of study provides actionable direction to malaria control programs by identifying gaps in coverage where interventions can be targeted. For example, the findings of this study illuminate that second residences of forest-goers are a critical intervention target in Aceh Province. While 76% of second residences were located in the forest or forest fringe, 100% of participants responded that these residences have either never been sprayed with indoor residual spraying, or that they do not know if these residences have been sprayed. Additionally, 97% of responses indicated the residences do not have any bednet. Detailed risk factor studies of this nature can also be used to determine specific malaria high-risk subgroups; in this case, the study has identified hole diggers for phone companies as a high-risk subgroup because they are often migrants, employed in large networks, and working away from settlements. Knowledge of high-risk subgroups and gaps in intervention coverage is essential for programs to effectively target interventions such as reactive case detection and vector control with IRS and bednets.

MERFAT Study Design

A case-control study is a classic epidemiological study design that is used to study a wide variety of diseases. In its simplest form, a malaria case-control study compares the characteristics of malaria cases to those of a population without malaria (controls).

MERFAT uses a health facility-based, case-control methodology in which both cases and controls are selected from individuals presenting at a health facility. This design is simple, easy to carry out and is designed for resource limited places with little malaria. In most settings, the case-control design will provide a valid comparison group for passively-detected malaria cases. The following section describes the components of the MERFAT design (Box 2).

Box 2. Components of MERFAT

The MERFAT design consists of the following:

1. Aims and programmatic questions to be answered
2. Geographical area of implementation
3. Case and control definitions
4. Inclusion and exclusion criteria for cases and controls
5. Malaria risk factors of interest
6. Sample size calculation

Aims of MERFAT

The standard MERFAT design aims to:

- Identify actionable risk factors for symptomatic malaria infection
- Profile HRPs in order to target them with interventions

Specific objectives for MERFAT can be developed in line with programmatic priorities. Examples of programmatic questions that can be answered using the MERFAT design include:

1. Do defined malaria HRPs exist? Can these groups be confirmed with data?

2. Where and when can malaria HRPs be targeted and/or accessed?
3. Can high-risk travel routes be identified for targeted surveillance?
4. Are existing interventions effective and what are the key gaps in malaria HRPs?
5. Are housing improvements likely to impact on malaria transmission and in which groups?
6. In what communities should the housing improvements be conducted?
7. Are environmental factors associated with having malaria?

Geographical Area of Implementation

The geographical area to implement MERFAT and the number of health facilities needed will be dictated by:

1. The number of malaria cases per health facility per month (see Figure 3).
2. The minimum number of cases and controls needed to identify risk factors – this will determine whether additional health facilities are needed.
3. Budget resources available for health facility staff. Use of existing health facility staff is more cost effective but in high-burden facilities, additional staff may be needed.
4. The populations for which the program requires information (where suspected HRPs are already identified).

Definition of Cases and Controls

The standard MERFAT design uses symptomatic malaria cases that are passively-detected at health facilities through the existing surveillance system. MERFAT controls are recruited from febrile individuals testing negative for malaria at health facilities (a 'test-negative' design). This design provides a basis for valid comparison by ensuring that the distribution of controls is the same as the source population of the cases with regard to treatment-seeking behavior. Figure 4 is a conceptual diagram of the selection and classification of cases and controls.

Case definition and selection

Cases should only include individuals who have been diagnosed with a *Plasmodium* infection and confirmed by microscopy, rapid diagnostic test (RDT), or another, more sensitive diagnostic test like loop-mediated isothermal amplification (LAMP) or polymerase chain reaction (PCR). All incident cases recruited for MERFAT should be interviewed at the health facility where they are diagnosed since follow-up may be difficult, especially for highly mobile populations.

Control definition and selection

Controls should only include individuals confirmed negative for a *Plasmodium* infection by microscopy, RDT or a more sensitive diagnostic (i.e., LAMP, PCR). Where reactive case detection is routinely carried out around index cases, a second set of controls selected from the community (community controls) may be recruited (Appendix 1).

Controls should be recruited in fixed numbers throughout the implementation period rather than matching by date of diagnosis. This will allow an estimate of how risk varies throughout the year in relation to environmental factors (e.g., rainfall, temperature and vegetation) and behavioral factors (e.g., travel patterns, outdoor work and use of preventive measures). Box 3 summarizes key considerations in matching cases to controls.

Box 3. Matching controls to index cases

Recruitment of controls should be restricted to reflect the expected age range and gender distribution of cases. For example, if all cases are males and over the age of 15 years, then the controls recruited from the health facilities should also be males over the age of 15 years. If one-third of cases are female, then a similar ratio of female controls should be recruited. This strategy is termed "restriction" or "frequency matching".

Another strategy sometimes used is to match controls to index cases based on individual case characteristics (e.g., age, gender, or time of diagnosis). However, this strategy is discouraged for the following reasons:
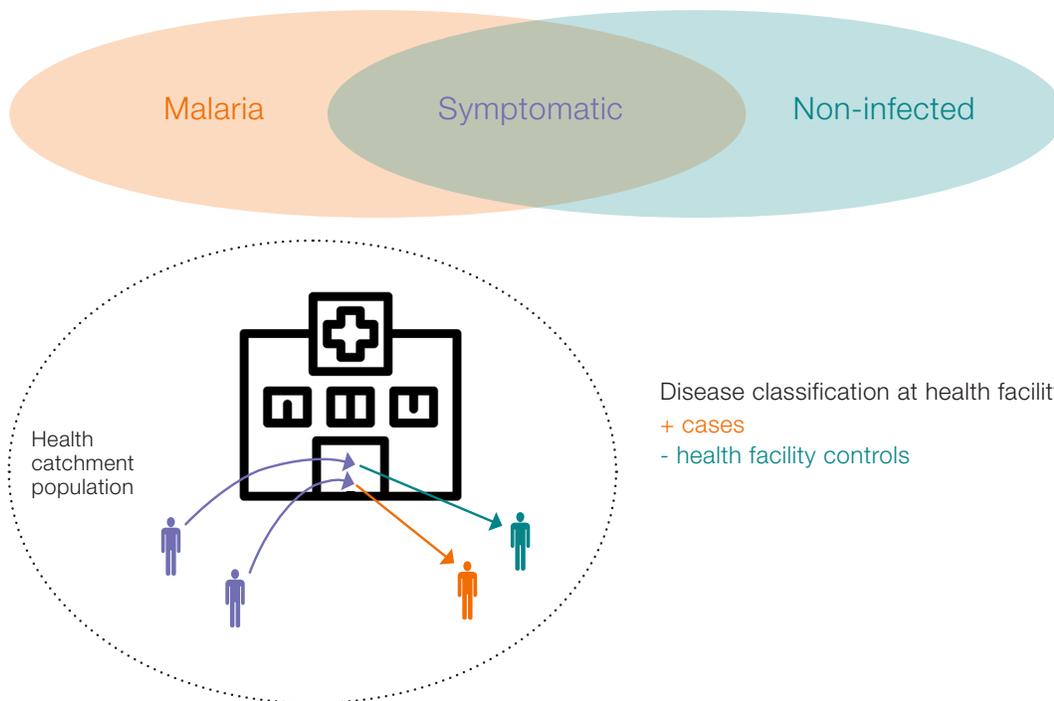
- It may be difficult to match cases and controls by calendar time (and other characteristics), thus leaving a large number of cases unmatched.
- Individual matching makes it impossible to differentiate the risk of an exposure (e.g., during travel) on the basis of the matching characteristics (e.g., gender and age).
- Age and gender can be measured and controlled for easily in the analysis using logistic regression or stratification, thus eliminating any need to individually match on these characteristics.

Figure 3. Disaggregated case table

#	Health Facility Name	# tested RDT/Mic.	Total	Gender		Age Group (years)				
				Male	Female	<5	5–14	15–29	30–44	45+
1										
2										
3										
4										
5										

Note: Disaggregating this table by month may help to plan for control recruitment targets.

Figure 4: Selection and classification of cases and controls

Malaria   Symptomatic   Non-infected

Health catchment population

Disease classification at health facility
+ cases
- health facility controls

Inclusion and Exclusion Criteria

Inclusion criteria are those characteristics that all participants must have to be included in the implementation of MERFAT. Exclusion criteria are those characteristics that disqualify individuals from participation. The same inclusion and exclusion criteria should apply to both cases and controls.

The objective is to ensure that the controls are representative of the same or similar population as the cases (i.e., a control would be detected and included as a case if he/she developed malaria). Box 4 summarizes the standard inclusion and exclusion criteria for the MERFAT design.

Box 4. MERFAT inclusion and exclusion criteria

Inclusion Criteria

1. Attending a selected health facility with suspected malaria
   - It is recommended that both cases and controls are recruited from individuals attending a selected health facility and presenting with fever, thus triggering malaria screening. This criterion will minimize selection bias and costs associated with control recruitment.
   - A second control set from the community may be recruited where reactive case detection is routinely conducted around the index case (Appendix 1).

2. Malaria test done
   - Both cases and controls should have a malaria test done by microscopy, RDT, and/or a more sensitive diagnostic test (i.e., LAMP or PCR). Cases should have a test positive result and controls should have a test negative result.

3. Willing and available to participate in MERFAT
   - All participants should be fully informed on the aims, risks and benefits of MER-FAT and have agreed to participate.

Exclusion Criteria

1. Prior diagnosis with malaria in the past 30 days
   - Cases that have received a diagnosis of malaria within the past month are likely to have a relapsed infection, rather than new infection. In areas where *P. vivax* transmission is dominant, it may be necessary to extend the period of prior diagnosis to 60 days, due to the higher probability of relapse infections.
   - Individuals who test negative for malaria, but have recently recovered from a malaria infection, can be considered part of the case population and so should not be included as controls.

2. Malaria chemoprophylaxis or treatment in the past 14 days
   - Individuals testing positive for malaria infection, but reporting recent chem oprophylaxis or treatment, are more likely to have a relapsed/uncured infection than a new infection.
   - Individuals testing negative for malaria infection, but reporting chemopro-phylaxis or treatment, are either not susceptible to infection (and therefore not at risk of being a case) or they may have had a recent infection.

3. Critically ill patients
   - Critically ill patients will need urgent medical care and may not be able to respond to the MERFAT questionnaire.

Malaria Risk Factors

The core MERFAT questionnaire measures exposure to a broad range of potential risk factors (Figure 5). This is typically assessed over a 30 day period, prior to diagnosis as a case or control. These factors can be related to three categories.
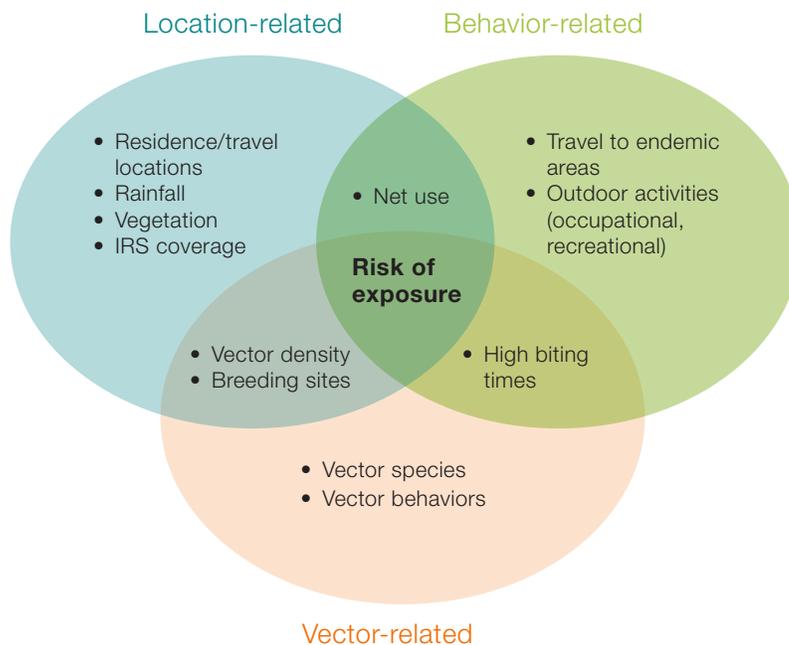
1. Behavioral risk factors related to human behavior (e.g., intervention use, occupation, leisure activities, or travel).

2. Location-related risk factors, including environ-mental factors associated with home or travel locations, rainfall, temperature and vegetation, as well as housing type (i.e., formal or informal structures). Nomadic pastoralists, for example, may live in informal structures that expose them to higher risk of mosquito bites compared to populations with more sedentary lifestyles.

3. Vector-related risk factors, including vector species and vector behavior (e.g., biting time and breeding sites) in the area where transmission takes place. If resources and entomological capacity are available, vector assessments around case and control households can be included to understand differences in peri-domestic transmission.

Sample Size Calculation

In order to determine the malaria risk factors with an acceptable level of certainty, a minimum number of cases is required. In elimination settings, there are likely to be few cases and reaching the required sample size may be difficult. In this situation, more controls should be recruited to increase the likeli-hood that the effect of a risk factor can be distin-guished. A detailed explanation of how to calculate sample size is provided in (Appendix 2).

Figure 5. Conceptual diagram of factors influencing risk of exposure to malaria

Location-related

Behavior-related

- Residence/travel locations
- Rainfall
- Vegetation
- IRS coverage

- Net use

Risk of exposure

- Travel to endemic areas
- Outdoor activities (occupational, recreational)

- Vector density
- Breeding sites

- High biting times

- Vector species
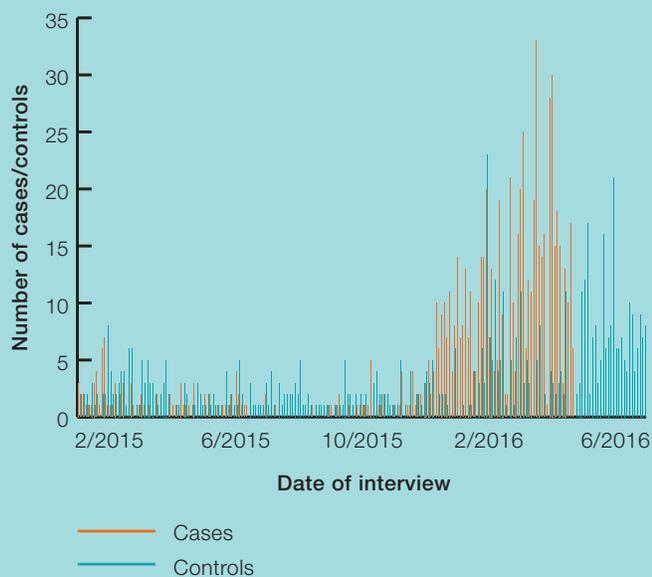- Vector behaviors

Vector-related

Box 5. Case Study: Identifying HRPs in Namibia

MERFAT was first piloted in Zambezi Region, northern Namibia between January 2015 and June 2016. The Zambezi Region borders several countries with higher malaria transmission. The Namibia Malaria Control Program was therefore interested in characterizing cross border movement and defining HRPs.

The pilot study covered two malaria transmission seasons. The first transmission season was low, with 96 cases and 199 controls, while the second had a large malaria outbreak and recorded 674 cases and 442 controls (Figure A). The case-control study revealed that males of all ages and children aged 5–14 years were more likely to be malaria cases.

Several programmatically relevant risk factors were identified after adjusting for education and socioeconomic status: cross-border travel, early migration in the transmission season, sleeping in tents or traditional houses, low bed net use, lack of IRS, engaging in outdoor activities at night specifically studying outside, and cow herding.

Using a technique to group high-risk characteristics, preliminary analyses identified three HRPs:

1. **Students**: Children aged 0–15 years, often males, who studied outside at night tended to have very low levels of intervention coverage and slept in tents or traditional houses.

2. **Mobile and migrant populations**: Adult males and cow herders who were characterized by high mobility and cross-border travel. They were more likely to sleep outdoors or in tents and were often not covered by IRS campaigns.

3. **Rural and remote residents**: Adults, both male and female, who tended to live further from health facilities and with lower education and socioeconomic status. They were likely to be missed by IRS campaigns, had very low net use, lived in traditional houses, and frequently engaged in agricultural work outside at night.

Figure A. Recruitment of cases and controls from February 2015 to June 2016

Component 1: MERFAT Planning

This section provides guidance and examples to develop the protocol, data collection instruments and budget for implementation of MERFAT. Editable versions of these documents are available upon request. Because all documents should be edited and adapted to the country context, the first step is to identify sections in the protocol, questionnaires and other documents that need to be adapted or where new sections should be created.

Funding for MERFAT Implementation

MERFAT can be implemented as an operational research project in collaboration with local or international academic institutions or nested within an existing surveillance system. Malaria programs may seek funding for MERFAT implementation from their governments, the Global Fund or other bilateral/multilateral donors. The MERFAT budget may include:

- Personnel costs – including costs for local Principal Investigator (PI), Field Coordinator, stipends or incentives for health facility staff (e.g., nurse and microscopist/laboratory technician) and hiring additional data collectors if required.

- Adaptation and pretesting of data collection tools (i.e., questionnaires and tablets)

- Training of field teams – including training on study procedures, refresher training for microscopists/laboratory technician and community sensitization

- Laboratory equipment and supplies (e.g., filter paper and PCR supplies for more sensitive molecular testing, if required).

- Travel (e.g., field coordinator supervisory visits, orientation visits, vehicle hire and fuel)

- Field supplies (e.g., tablets and/or printing, air time, office supplies)

- Institutional Review Board (IRB) costs (if required)

- Technical support for data analysis, report writing and dissemination

- Administrative costs for participating health facilities (e.g., charging laptops and telephone charges)

- Other costs determined by local context

Formation of MERFAT Study Team

Implementation of MERFAT can be led by a PI from a local public health research institution or by a technical NMP staff member. The PI should be supported by NMP staff, a Field Coordinator and a data manager/analyst. These positions can often be filled through part-time effort and/or by graduate students from local public research institutions. The MERFAT implementation team should take the lead in adapting the protocol and oversee the technical and operational aspects of the project.

Adapting the MERFAT Design

All materials will need to be adapted to a particular study area and in some settings, the design may be adapted to focus on particular populations or purposes. We recommend adapting the MERFAT design with technical assistance from UCSF MEI or other technical partners.

Step 1. Review existing case data and compile information on HRPs

The first step in adapting the design is to review available information from:

- Monthly data from health information management systems (HMIS

- Patient registers, and

- Local malaria experts at the community level (i.e., health facility level or lower)

This can be done as a part of *Module 1: Planning Targeted HRP Surveillance and Response*.

Figure 6 outlines the key questions and data inputs that should be considered. This will help to tailor the design of MERFAT and staffing decisions.

Step 2. Review MERFAT aims

While the standard MERFAT design captures a broad range of risk factors, it may be useful to modify the aims if there are known characteristics of HRPs, based on prior studies or expert knowledge. Programs may also wish to:

- Ensure that environmental risk factors can be investigated and geographical clustering explored

- Confirm a suspected HRP (and remove other exploratory questions), or
- Focus investigation into behavioral risk factors or intervention gaps in a known HRP (such as mobile and migrant populations).

To ensure unbiased implementation of MERFAT, it is best to seek technical support from partners when making changes to the design. Appendix 3 outlines some tailored MERFAT design options based on specific risk factors.

Figure 6. Key questions and inputs for organizing design of MERFAT

Data source	Activities	Decision points	Actions
HMIS	Calculate number of cases by: • Health facility • Month • Species • Travel & importation status (if available)	How many health facilities need to be included to reach the minimum sample size?	Determine the geographical area for MERFAT implementation
		How long will implementation need to extend to reach the minimum sample size?	Propose timeline for data collection
		Are there sufficient cases to analyze the results by species?	Calculate minimum sample size for all species or separately for each species
Registry review	Calculate number of individuals tested and cases per month, by: • Age • Gender • Occupation (if available) • Location (if available) • Travel and importation status (if available)	What is the historical age and gender distribution of cases? Will the monthly control recruitment targets at health facilities be met?	Set the monthly control recruitment targets for each health facility based on age and gender-specific caseloads
		What are the expected rates of malaria importation? From where are the cases likely to be imported?	Use occupation, travel and/or location data to inform MERFAT aims and questionnaire design
Expert opinion	Consult with local experts about: • Whether there are known/suspected HRPs in the proposed area, and how common they are • Whether HRPs vary throughout the proposed area • Levels of access to treatment of different HRPs	Is <10% of the population likely to fall into a high-risk category?	Increase the sample size in order to detect small groups, if needed
		Are HRPs likely to vary across the implementation area?	Split the implementation area into homogenous zones if HRPs vary
		Are there likely to be HRPs who do not attend health facilities?	Consider using time location sampling or a peer referral method to access specific, hard-to-reach HRPs

Step 3. Identify the geographical area for implementation

The catchment population of selected health facilities will typically determine the MERFAT implementation area. Selecting specific districts and all health facilities (or a sample) within them is recommended. The selected area should meet the needs of the malaria program, capture the required sample size and be operationally feasible. Box 6 summarizes the considerations for selecting the geographical area for MERFAT implementation.

It should be noted that the geographical catchment area of a health facility does not always correspond to the true catchment area. For example, health facilities located near border posts may capture mobile and migrant populations (MMPs) who are in transit.

Box 6. Considerations for where to implement MERFAT

- Programmatic area for which information is needed – this can be large, such as a health district, or small, such as border areas
- Environmental risk factors (e.g., rainfall and vegetation) and how they vary across the area
- Number of confirmed cases of malaria in the area per year and number of health facilities that generate them
- Resources to implement MERFAT – it may be more cost-efficient or cost-effective to expand the geographical area (in order to increase the number of cases) or to extend implementation over several transmission periods
- Whether the expected HRPs are likely to have similar or very different characteristics
- Whether the area is contiguous or is comprised of several districts or enumeration areas separated spatially

Step 4. Calculate the sample size

In some circumstances, the recommended sample size may need to be inflated if:

- Sub-group analyses is required - this can be by malaria species or occupational groups.
- The expected prevalence of some high-risk characteristics (e.g., occupational groups or travel) is less than 20% in the general health-seeking population.
- The MERFAT area is large and expected high-risk characteristics are only present in some areas

More details on sample size calculation are provided in Appendix 2.

Step 5. Choose the duration of the study

Duration of MERFAT implementation will have budget implications and will depend on whether one transmission season will be sufficient to achieve the minimum sample size within the proposed study area. Given potential variation in transmission between years, be prepared to seek funding if data collection needs to be extended to meet the sample size.

Step 6. Decide what type of ethical review is needed

National guidelines from the Ministry of Health as well as requirements from implementing or technical partners will determine whether ethical approval will be required to implement MERFAT. If this is required, a section should be included in the protocol that details what type of review will be obtained as well as procedures around informed consent and data confidentiality.

Adapting the MERFAT Questionnaire

The standard MERFAT questionnaire provides a core set of questions covering different aspects of malaria (Figure 7). Malaria programs will need to adapt the questionnaire to fit their setting. Programs may choose to remove or alter some questions. Modifications to the questionnaire should be done with technical assistance. The following steps should be taken to adapt the standard MERFAT questionnaire to the local context.

Figure 7: Overview of the MERFAT questionnaire

Recruitment strategy & Socio-demographics

Clinical data	Recruitment
Demographics	Occupation
Residence location	

Household data

Number of residents	Environmental characteristics
Housing characteristics	
IRS history	Forest workers guest/family*

Knowledge & prevention

Net use	Prior treatment seeking
Malaria knowledge	

Outdoor risk activities

Type of activity	Frequency
Duration	Protection
Sleeping outside	

Domestic travel

Destinations	Transit points
Travel network	Seasonality
Acceptable interventions	Mode of Travel

Cross-border travel

Destinations	Transit points
Travel network	Seasonality
Acceptable interventions	Reason for travel
Mode of travel	

Forest travel

Destinations	Transit points
Mode of travel	Travel network
Acceptable interventions	Seasonality

Step 1. Review core questions and responses

These questions usually remain unchanged but the language may need to be modified to fit the setting. If *P. vivax* is dominant, consider extending the period of exposure for key risk factors from 30 to 60 days.

The response sets will need to be tailored to each setting. For example, names of nearby countries, health facilities and typical materials used in housing construction should be inserted. It is important to develop a very detailed list of occupations in each

setting, particularly those suspected to be common or potentially high risk. This can be determined by a formative review, as described in *Module 1, Formative Assessment for Planning Targeted HRP Surveillance and Response*.

Step 2. Replace all administrative lists with country-specific sub-divisions (ODK only)

The standard Open Development Kit (ODK) version of the MERFAT questionnaire is developed to filter responses by administrative levels 1 (region/province), 2 (district), 3 (sub-district), and 4 (village). For this to work, a .csv file that specifies the country administrative levels needs to be made. This file should be adapted, keeping the column headers titled exactly as they are in the ODK questionnaire.

Step 3. Add or remove additional modules

Additional modules may be added or removed from the standard MERFAT questionnaire. For example, a module on forest travel may be included where transmission in this setting is suspected or confirmed.

Step 4. Translate questions into the local language

Translate the questions in the paper questionnaire and text in the 'label' and 'hint' columns in the ODK form into the local language ('label::X', 'hint::X'). This should be translated as closely as possible by two separate translators and then compared. The final version should be back translated to English and compared against the original text. In the ODK form, you can switch between languages.

Step 5. Generate a photobook for each Interviewer

When asking questions related to types of housing (e.g., construction materials of walls), household environmental factors (e.g., presence of long grass) or types of animals present, it is recommended to have pictures for Interviewers to show to the respondents. This improves the reliability of self-reported data for these questions.

Step 6. Pilot the questionnaire

The questionnaire should be piloted with at least five people of the same background as potential participants. This will help to ensure that questions are understandable and the correct data are being collected.

Component 2: MERFAT Data Collection

Data collection should be planned to cover at least one full transmission season, but may be extended to increase the sample size or carried out regularly to inform ongoing surveillance. Implementation of MERFAT will be successful only if each team member understands and follows correct data collection procedures. The following section describes the staffing requirements and procedures for data collection.

Staff Roles and Responsibilities

Project staff must adhere to the ethical principles and standards when conducting the survey. Most importantly, they must respect and protect the privacy, confidentiality, and autonomy of participants. In addition, project staff should conduct themselves in a professional manner when interacting with participants, fellow staff members, and the general public.

A dedicated team of field staff is required to successfully implement MERFAT. The field staff include the following positions:

- Field Coordinator
- Interviewers
- Health facility nurse

The roles and responsibilities of these positions are described below. A detailed list of responsibilities of the field team is found in Appendix 4.

Field Coordinator

The Field Coordinator is responsible for the day-to-day management of all survey activities, including:

- Data collection (interviews and biological specimens)
- Quality assurance
- Correct management of data and records

The Field Coordinator is also responsible for direct supervision of other field staff, leading regular debriefing calls, and developing progress reports. Extensive in-person supervision of field staff is critical in the first several months to ensure adherence to protocols and high quality data collection.

Interviewer

Interviewers will be responsible for:

- Screening cases and controls for eligibility
- Administering informed consent
- Interviewing all cases of malaria and eligible controls
- Documentation of all data collection forms
- Ensuring quality of interview data
- Storing all biological samples

Interviewers must report any problems to the Field Coordinator. In low burden areas, existing health facility staff (either nurses or designated malaria officers) can be recruited as interviewers. In high burden areas, the number of malaria cases may exceed capacity of the existing staff, and a designated interviewer may be hired to assist with the data collection.

The time needed for one interviewer to enroll one survey participant is likely to be about 45 minutes, including eligibility screening, informed consent, survey interview and malaria testing. This estimate should be used to determine the number of staff required to conduct MERFAT procedures at each health facility.

Health Facility Nurse

Nurses are responsible for the following:

- Testing individuals presenting to a health facility with symptoms of malaria
- Creating dried blood spots (DBS) for further testing
- Coordinating with Interviewers to ensure correct labeling and storage of DBS
- Treating and referring individuals testing positive for malaria according to national guidelines

Testing and treatment is guided by country-specific guidelines. The results of the tests create the pool of potentially eligible cases (positive for malaria) and controls (negative for malaria).

Training of Field Staff

The field team should be trained on the aims of MERFAT and SOPs, such as selection of eligible participants, obtaining informed consent, handling specimens, and administering the questionnaire. The training should also include ethical principles and standards in conducting the MERFAT procedures, such as respect for the privacy and autonomy of the participants and safeguarding confidentiality. Training should include a refresher course on malaria diagnosis for the nurse and microscopist/laboratory technician. The duration of the training may vary depending on experience of the staff, but generally one week is considered sufficient. On-the-job training should be continued during supervisory visits.

Preparation of Materials

Interviewers and nurses must have the necessary materials on hand to complete their daily tasks. Materials required for MERFAT implementation are listed in Box 7. All the materials and supplies should be made available before implementation starts. Unique participant identification codes should be prepared in advance. Barcodes for labelling samples and data collection forms should be pre-printed in advance. Appendix 5 provided details on how to generate unique participant IDs and pre-print the barcodes.

Implement Data Collection

Figure 8 presents an overview of MERFAT data collection procedures, which include the following activities:

1. Case selection and eligibility screening
2. Control selection and eligibility screening
3. Administering informed consent
4. Assigning the participant ID and labeling materials
5. Administering the survey questionnaire
6. Collecting blood samples and carrying out laboratory procedures
7. Reviewing and uploading survey forms

Table 1 summarizes the key steps during MERFAT data collection.

Step 1. Case selection and eligibility screening
Staff member: Interviewer

Upon arrival to the health facility, patients with suspected malaria will be tested according to country-specific national guidelines. Typically, this includes all febrile individuals. All patients who test positive for malaria by microscopy or RDT (confirmed cases) at any of the participating health facilities should be assessed against the eligibility criteria laid out in Appendix 5.

Material required
- Participant folder (containing two copies of the Informed Consent form and eligibility screening form)
- Health Facility Case Line List (Appendix 7)

Procedures
1. Complete the paper-based case notification form (if applicable).
2. Complete the eligibility screening form (Appendix 5) to determine if the case is eligible to participate in MERFAT implementation.
3. If the case is eligible to participate, enter patient name and details on the Health Facility Case Line List.

Step 2. Control selection and eligibility screening
Staff member: Interviewer

Upon arrival to the health facility, patients with suspected malaria will be tested according to national guidelines, which typically includes all febrile individuals. All persons who test negative for malaria by microscopy or RDT at any of the participating health facilities should be assessed against the control eligibility criteria laid out in Appendix 5.

Material required
- Participant folder (containing two copies of the Informed Consent form and eligibility screening form)
- Health Facility Control Line List (Appendix 8)
- Monthly Control Recruitment Tracker (Appendix 9)

Procedures
1. Complete the eligibility screening form to determine if the control is eligible to participate in the study. This form includes a question on whether recruitment targets for the patient's

specific age and gender have been met for the current month.

2. If the control is eligible to participate, enter patient name and details on the Health Facility Control Line List.

Step 3. Administering informed consent

Staff member: Interviewer

It is important that each eligible individual invited to participate fully understands all procedures and how their samples and data will be used. The process of informed consent is a necessary ethical procedure preceding any data collection, and no samples or data should be analyzed if consent is not provided.

Even when MERFAT is performed as part of routine surveillance, as opposed to a research activity, it is important that participants have a clear understanding of the procedures involved and provide informed consent to participate. See Appendix 10 for a sample informed consent form.

Material required
• Participant folder (containing two copies of the In-formed Consent form and eligibility screening form)

Procedures for administering informed consent are summarized in Box 8.

Box 7. Checklist of materials required for MERFAT

Survey Coordinator
☐ Master case recruitment line list
☐ Master control recruitment line list
☐ Extra DBS cards
☐ Extra paper questionnaires
☐ Extra pens
☐ Extra forms (line lists, screening, informed consent, refusal)
☐ Extra tablet (charged) and charger
☐ Coolbox with ice to transport samples to centralized location
☐ Extra refreshments (if applicable)

Interviewer
☐ Health facility case recruitment line list
☐ Health facility control recruitment line list
☐ Monthly control recruitment tracker
☐ Pre-printed participant ID stickers (barcode stickers if possible)
☐ Tablet (charged) and charger
☐ Paper copies of questionnaire

☐ Eligibility screening forms
☐ Informed consent forms
☐ Refusal forms
☐ Copies of approval letters from the bioethics committee and administrative approval
☐ Refreshments for participants

Nurse
☐ DBS cards
☐ Gloves
☐ Alcohol swabs
☐ Lancets, syringes, needles
☐ Cotton or gauze
☐ Biohazard plastic bag (red)
☐ Plastic bag for other trash (black)
☐ Sharps container
☐ Pencils, pens, and permanent markers (sharpies)
☐ Clear plastic zip bags for samples
☐ Desiccant for zip bags
☐ Drying racks for slides and DBS
☐ Refrigerator (in health facility)

Figure 8. A systematic flowchart of case/control classification and data collection procedures
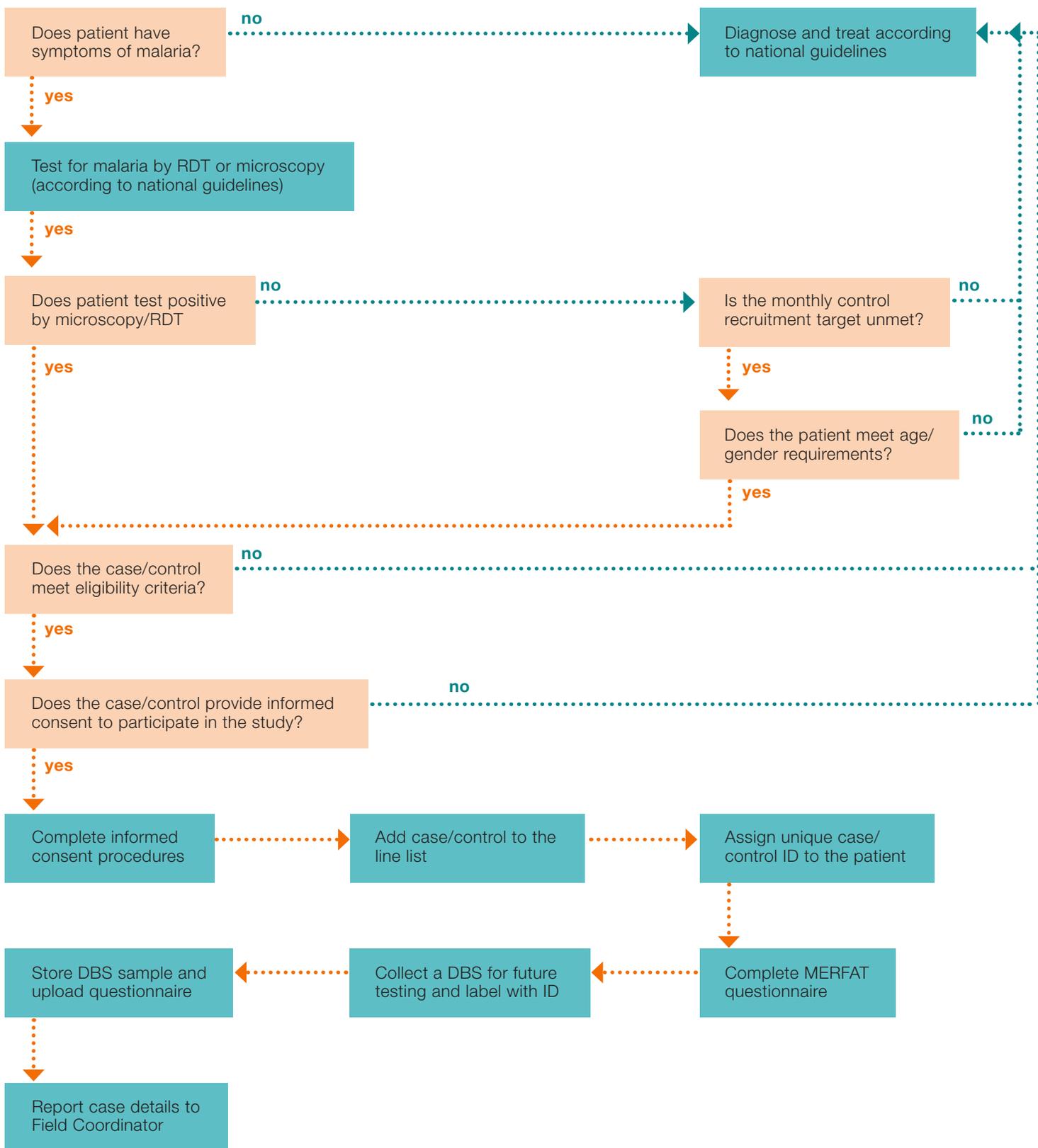
Table 1. Key steps and during the data collection phase

Before data collection	• Visit all health facilities to be included and orient all nurses/existing staff to their role in the study • Prepare materials • Set up interview area
Ongoing, for each participant	• Potential participant is tested for malaria according to national guidelines (microscopy or RDT) • Forward participant to interview area • Check eligibility status • Assign participant ID • Administer informed consent • Administer the questionnaire • Create slides and DBS • If malaria positive, administer treatment according to national guidelines • Provide prevention materials (if applicable) and thank participant
Daily	• Upload data to central server from tablet (if applicable) • Daily planning and debriefing call • Review all records of each participant, separate information sheets and blood samples and store in a safe location • Store all survey equipment and documents/forms in a secure, restricted-access location • Charge tablet (if applicable)
Weekly	• Data checks to summarize recruitment numbers and any refusals • Pick up of all laboratory samples and transport to a centralized storage unit • Pick up of documents/forms and transport to centralized storage unit • Double enter paper questionnaires (if applicable)

Box 8. Informed consent procedures

- Using the script on the case/control informed consent form (Appendix 10) explain the purpose of MERFAT and its procedures and invite the patient to participate in blood testing and answering the survey questionnaire.

- If the patient provides consent, have them sign both copies of the case/control consent form. If the participant is a youth under 18 years, informed consent will be obtained from a parent or guardian.

- Add a tick in the appropriate box on the 'Health facility case line list" (cases only) or the 'Health facility control line list' (controls only).

- Affix a barcode to one copy of the informed consent. Keep this copy and write in the ID number on the second copy to give to the participant.

- Write the participant ID on the case (or control) line list.

- Add a tick next to the appropriate age and gender category in the 'Monthly Control Recruitment Tracker" [Controls only].

Patients who decline to be interviewed are not recruited to participate in MERFAT. Refusal to participate does not affect access to health care.

Step 4. Assign Participant ID and label materials

Staff member: Interviewer

If the patient agrees to participate in MERFAT, assign them an identification (ID) code. This code will be used to link blood samples to epidemiological data. The codes should therefore be unique and entered correctly. The best practice is to use barcode IDs, which can be pre-printed before the study and scanned into the tablet to avoid mistakes in data entry. Box 9 summarizes the barcoding and labeling procedures.

Material required
- Pre-printed barcode IDs
- A felt tip pen to mark any samples missing an ID

> **Box 9. Barcoding and labeling procedures**
>
> Use the next available participant ID, specific to a case or a control and place barcodes as follows:
>
> a. Place a single barcode on the informed consent form.
> b. Write the barcode identifier and date the patient presented on the second copy.
> c. Place a single barcode on the case or control line listing.
> d. Write the barcode ID or place a single barcode sticker on the positive micros-copy slide/RDT to indicate that the slide is now tracked. Ensure that the pen ink is permanent.
> e. Place the double barcode on the outside of the DBS sample.

Step 5. Administer the survey questionnaire
Staff member: Interviewer

The survey questionnaire is the main data collection tool for MERFAT. The questionnaire can be admin-istered in paper form or electronically using a tab-let. Training materials and tips on how to conduct interviews are available from MEI. The interviewer should administer the questionnaire calmly and avoid making the respondent feel pressured or uncom-fortable. Appendix 11 provides guidance on how to conduct interviews.

Keep in mind that people who are invited to participate in the MERFAT as cases or controls have sought treatment because they feel ill. Eligible patients should be prioritized accordingly and those who are critically ill should not be interviewed.

Material required:
- Tablet (make sure the battery is charged)
- Paper copy of questionnaire and pen as back-up
- Participant folder (containing two copies of the Informed Consent form and eligibility screening form)

- Photobook with pictures of relevant housing/environ-mental variables (e.g., construction materials, different types of vegetation)

Procedures
1. Complete the interview on tablet or on paper-based questionnaire.
2. When asking the questions about specific house construction materials for walls and different types of vegetation, pick out the (correct) pictures from the photo book. This will confirm participants' understanding of the questions related to housing risk factors and surrounding environment. At the end of the interview, thank the participant and escort them to the nurse to take a DBS.

Step 6. Collect blood sample and laboratory procedures
Staff member: Nurse

Material required
- Filter paper and envelope for DBS
- Lancet
- Gloves
- Alcohol swab
- Slides

Procedures
1. Collect the following: 1) DBS sample; 2) slide. Detailed procedures for these activities are available in Appendix 12.

Step 7. Review and upload survey forms
Upon completing the above procedures, immediately do a full review of all forms to ensure that:

1. Participant IDs are correct on the tablets/paper questionnaire, blood samples, line listing and informed consent forms
2. Informed consent forms are signed by the participant or guardian (if under 18 years of age)
3. Tablet/paper questionnaire is completely filled out

Once all the forms are confirmed to be correctly completed, the tablet form should be uploaded to a centralized server. This may be done immediately or at the end of the day.

All paper forms should be stored together in the Participant Folder. As soon as the DBS is dry, it should be packed in a labeled plastic bag with desiccant and stored in a freezer.

Documentation and Data Management

Documentation provides a written account of the processes and activities undertaken as well as decisions made during the implementation of MERFAT.

This section provides guidance on data cleaning, governance, storage and preparations for data analysis. Table 2 summarizes MERFAT data documentation and storage procedures.

Table 2. Description and storage of key MERFAT documentation

Name	Format	Description	Storage
Eligibility forms	Paper	• Eligibility decision tree for cases/controls. • Should be completed for each individual tested for malaria.	• Kept in the participant folder. If the person is NOT eligible to participate, will be stored in a separate folder.
Informed consent	Paper	• Informed consent forms to explain the study. • Each participant should have two signed copies.	• One copy (with the barcode ID) will be kept in the participant folder. The other copy (with ID written on it) will be given to the participant.
Case line lists	Paper *Master case line lists* should also be entered into an excel spreadsheet.	• *Health facility case line lists* list all eligible cases each participating health facility. • *Master case line lists* include all eligible cases at all participating health facilities.	• *Health facility case line lists* must be maintained in the study binder at the health facility. • *Master case line lists* are maintained by the study coordinator and entered into an excel sheet on a weekly basis.
Control line lists	Paper *Master control line lists* should also be entered into an excel spreadsheet.	• *Health facility control line lists* list all eligible controls at each participating health facility. • *Master control line lists* includes all eligible controls at all participating health facilities.	• *Health facility control line lists* must be maintained in the MERFAT binder at the health facility. • *Master control line lists* are maintained by the study coordinator and entered into an excel sheet on a weekly basis.
Survey Questionnaire	Tablet (or paper)	• Data collection instrument	• All completed forms must be uploaded to a centralized server at the end of each day.

The following general procedures should be followed in order to ensure proper management and storage of all data and samples:
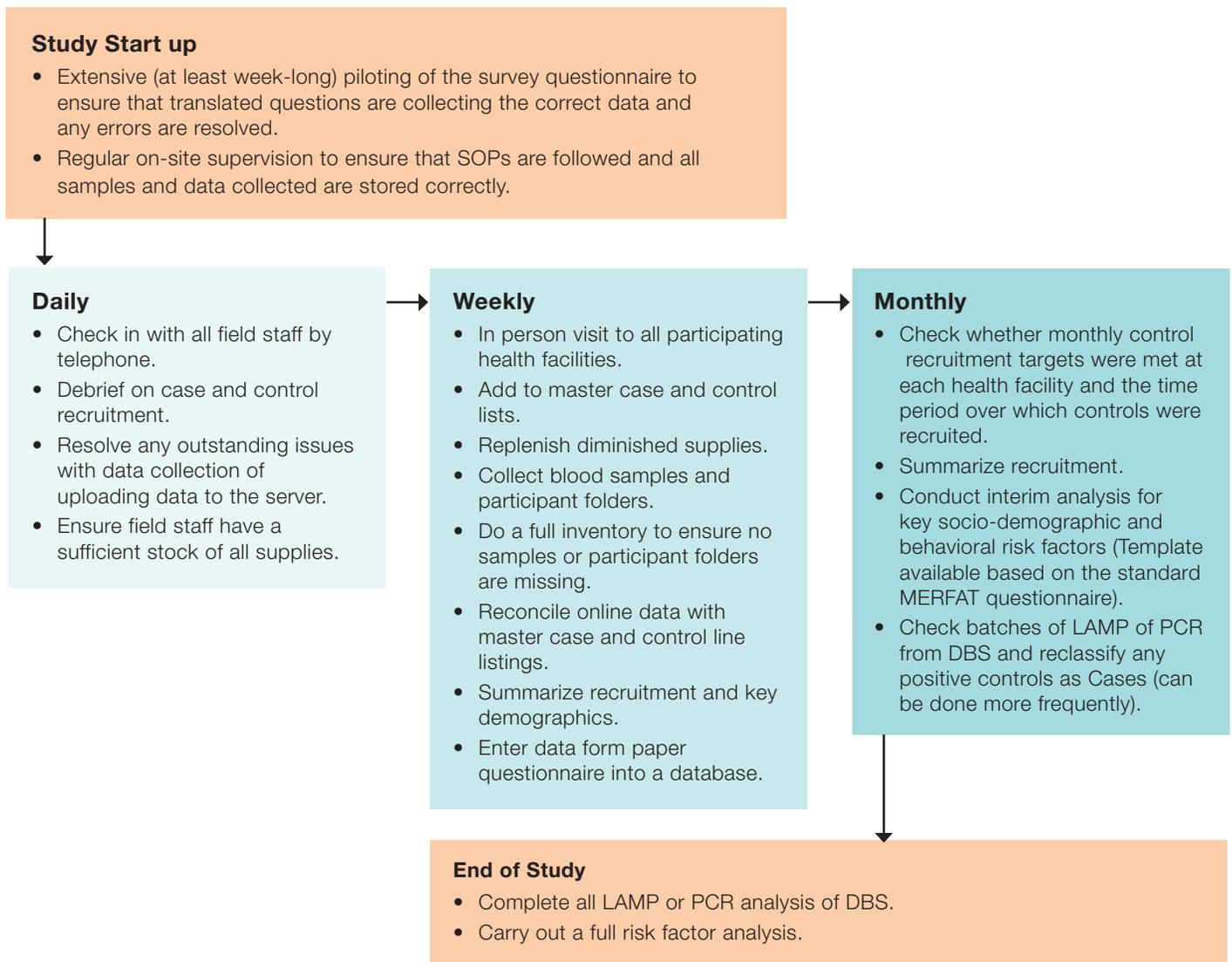
The Interviewer should:

- Collect the original slide/RDT.
- Ensure the DBS is properly dried.
- Organize the samples (Appendix 12).
- Ensure all paper documents (eligibility screening form, informed consent form, and case notification form, if applicable) are correctly filled out and stored together in the Participant folder.

The Field Coordinator should:

- Copy entries from the health facility case line listing into the master case line listing on a weekly basis.
- Pick up the slides/RDT and DBS every week and transport them to a centralized laboratory for further testing.

Close coordination between the Field Coordinator and all Interviewers is essential. The Field Coordinator is responsible for reconciling all survey questionnaires submitted electronically with paper line lists from the health facilities and collected samples. Figure 9 outlines the data management procedures that the Field Coordinator should carry out regularly.

Figure 9: Regular MERFAT data management procedures conducted by the Field Coordinator

Study Start up
- Extensive (at least week-long) piloting of the survey questionnaire to ensure that translated questions are collecting the correct data and any errors are resolved.
- Regular on-site supervision to ensure that SOPs are followed and all samples and data collected are stored correctly.

Daily
- Check in with all field staff by telephone.
- Debrief on case and control recruitment.
- Resolve any outstanding issues with data collection of uploading data to the server.
- Ensure field staff have a sufficient stock of all supplies.

Weekly
- In person visit to all participating health facilities.
- Add to master case and control lists.
- Replenish diminished supplies.
- Collect blood samples and participant folders.
- Do a full inventory to ensure no samples or participant folders are missing.
- Reconcile online data with master case and control line listings.
- Summarize recruitment and key demographics.
- Enter data form paper questionnaire into a database.

Monthly
- Check whether monthly control recruitment targets were met at each health facility and the time period over which controls were recruited.
- Summarize recruitment.
- Conduct interim analysis for key socio-demographic and behavioral risk factors (Template available based on the standard MERFAT questionnaire).
- Check batches of LAMP of PCR from DBS and reclassify any positive controls as Cases (can be done more frequently).

End of Study
- Complete all LAMP or PCR analysis of DBS.
- Carry out a full risk factor analysis.

Component 3: Data Analysis

Analysis of the data collected from MERFAT should be geared towards the primary objective of identifying actionable risk factors for symptomatic malaria infections in order to target interventions. The analysis should follow clear steps to:

1. Define recruitment patterns
2. Map patterns of malaria
3. Characterize cases and controls
4. Identify key actionable risk factors

Steps 1 and 2 should be conducted on a regular (i.e., biweekly) basis in order to check whether recruitment of controls is on target and to identify any emerging risk factors. A more complex and multi-variate analysis of risk factors, or analysis to account for matching in the study, can be conducted with technical assistance.

Step 1. Recruitment tracking

To track recruitment, perform the following analysis:

- Calculate the number of cases and controls recruited into the study to date and compare them against the recruitment target.

- Generate a histogram to observe the number of cases and controls (separate bars) recruited by week within the study period.

- Compare the patterns of case and control recruitment to identify when the case burden is higher and malaria transmission is more intense.

Note that the number of cases will fluctuate with malaria transmission (as well as any other temporal factors influencing treatment-seeking, such as holidays) while the number of controls should remain relatively stable.

Step 2. Characterize cases and controls

Calculate the number and proportion of cases and controls that have specific characteristics of interest. This can be profiled by:

1. Demographic characteristics (e.g., age, gender, residence location)

2. High-risk behaviors or activities (e.g., occupation, travel/forest exposures, housing, sleeping outdoors).

Table 3 outlines the key risk factors that can be profiled in a MERFAT analysis. These numbers and statistics can be visualized using histograms.

Category	Variable Name	Rationale
Socio-demographics	• Age • Gender • Citizenship • Main occupation	Profile sub-populations
Risk activities	• Outdoor activities at night • Slept outdoors in past 30 days • Slept within 20m of cattle	Identify risk behaviors to target
Housing and malaria prevention	• Net use (frequency) • Net slept under prior night • IRS • Traditional vs Modern housing • Open vs Closed housing	Identify intervention gaps
Travel and mobility	• Intervention use • Duration lived at residence • Second residence • Domestic travel • Cross-border travel • Worked/slept in forest	Profile sub-populations/ Identify risk behaviors/ Identify intervention gaps

Step 3. Identify key actionable risk factors

In order to identify key risk factors to inform programmatic action, the odds (or risk) of exposure in cases is compared to controls. If cases are more likely to have been exposed to a particular behavior or activity than controls, then we consider that it might be important in malaria transmission.

If the distribution of any factors have been restricted by design in controls (e.g., age, gender, or by setting a health facility recruitment target), these must be controlled for at the analysis stage. This is because the restriction factors are not representative of the population and may change observed risk factor associations. The standard MERFAT analysis will control for the health facility where recruitment took place, age and gender while calculating the odds ratios for all of the above key risk factors and an indication of whether this odds ratio is likely to be associated with increased or decreased risk of malaria.

There are likely to be additional, potentially important risk factors that are not captured in Table 3. A complete analysis can be conducted using standard statistical software packages, such as STATA, SPSS or R. Technical assistance for this analysis can be sought out as needed.

Step 4. Map patterns of malaria

The standard MERFAT questionnaire will collect information on the residence of cases and controls, down to the village level. These village locations can be geolocated, or assigned to a specific longitude and latitude. Village coordinates may already be available from national census documents or other databases, such as school databases or from malaria program reactive case detection activities. If the coordinates are not available, a field team member may have to go out with a GPS-enabled device, such as a smartphone, tablet or tracker, to capture these coordinates. It is important to ensure that each village is assigned exactly the same coordinates in the database.

The number of cases and controls, village name and coordinates can be mapped using a GIS program. Technical assistance can be sought as needed.

Appendix 1: Community-Based Controls

The standard MERFAT design uses health facility-based controls. In some situations, recruitment of community-based controls may be recommended, such as where reactive case detection is routinely conducted and when a detailed examination of specific occupations or behaviors is required. Community controls may also be recruited where several malaria cases are reported from one or two geographically close villages.

Controls living in close proximity to index cases are also likely to be more similar in terms of socioeconomic status, intervention coverage, occupation, and housing, thus underestimating any risk associated with these broader risk factors.

Advantages of community-based controls

- Community-based controls are selected from the same village or neighborhood as the cases and are more likely to have similar access to health care.

- Community-based controls may provide a better comparison for treatment-seeking and other types of behaviors that may be affected by any illness.

- A control group (randomly) selected from the same community as cases can help ensure that controls are a representative sample from the same source population.

Disadvantages of community controls

- Controls living in close proximity to index cases are likely to be similar in terms of socioeconomic status, intervention coverage, occupation, and housing, thus underestimating any risk associated with these broader risk factors.

- Including a set of community-based controls will add cost and require a more complex analysis to incorporate matching at the village or neighborhood level.

Appendix 2: Calculating Sample Size

In the analysis, risk factors for malaria are expressed as associations between an exposure and being a case or a control. This is quantified through an odds ratio (OR). For example, if 40% of cases have a risk factor but only 20% of controls have it, the cases can be said to have 2.67 times greater odds of the risk factor than the controls. In order to say this with an acceptable level of certainty, a large enough number of cases is needed.

In general, a reasonable minimum sample size will be 92 cases and 92 controls in order to have adequate power (80%) to distinguish an OR of 2.67, assuming a 20% prevalence of a risk factor in controls and 40% prevalence in cases.

In elimination settings, there are likely to be few cases and reaching the target sample size may be difficult, especially when there are low prevalence risk factors. In this situation, more controls (up to 4) per case should be recruited in order to increase the power and decrease the number of cases required to ~65. Table 1 demonstrates how adding controls can reduce the number of cases required.

When to increase the sample size

In many circumstances, the minimum sample size will need to be increased to account for the following:

- **Lower prevalence exposures**: If the suspected prevalence of some high-risk characteristics (e.g., occupational groups or travel) is less than 20% in the general population or a lower effect is of interest, a higher sample size will be required.

- **Sub-group analyses**: If you are planning to do sub-group analysis by malaria species or occupational group, you will need to increase your sample size. To achieve the same power (80%) for each sub-group, you will need to capture the total sample size in each sub-group.

- **Non-contiguous area**: Where implementation of MERFAT covers multiple health facilities over a non-contiguous area, a design effect must be added to inflate the sample size, to account for the fact that many exposures (e.g., travel behaviors) may cluster geographically. A minimum design effect of 1.5 is recommended, and its effect on sample size can be explored in the MERFAT Sample Size Calculator (in R), which is available upon request.

- **Matching**: If controls are matched on some factor (e.g., village of residence), then a higher design effect should be used to account for added correlation between cases and controls.

- **Focal risk factors**: If the MERFAT implementation area is large, and high-risk characteristics are only expected to be present in certain areas, the area should be split into homogenous strata and desired sample size for each strata obtained. The total sample size will include the desired sample size from each strata.

A MERFAT Sample Size Calculator is available (in R) upon request to explore the effect of changing these assumptions on the required sample size.

Table 1. Sample size calculations for unmatched case-control studies, assuming 20% prevalence of exposure of interest (cases) and 40% prevalence (controls), with 80% power and alpha = 0.95. Community controls are assumed to come from the neighborhood of cases and have a correlation of 0.3.

Cases: control Ratio	Odds ratio	Sample size			Total sample
		Cases	Health facility controls	Community controls	
1:1	2.67	92	92	(125)	184
1:2	2.67	72	144	(172)	216
1:3	2.67	65	195	(216)	260

Appendix 3: MERFAT Design Options for Specific Risk Factors

Risk factors of interest	Programmatic relevance	Study design options
Environmental factors	Malaria may cluster into geographical 'hotspots' that are related to topography, vegetation and other environmental factors. These may influence vector density and habitat availability, resulting in transmission foci.	If more than one health facility is included in the implementation of MERFAT, ensure a spatial analysis can be conducted by assigning controls to health facilities based on probability proportional to size. This will ensure that the distribution of the control population in space matches the true population distribution. A risk mapping software like DISARM may be used to model risk factors and predict malaria incidence using information on the underlying population distribution instead of a control series.
Forest malaria	When all malaria transmission is forest-related, the objective may be to identify whether there are HRPs or high-risk behaviors within forest-going populations.	Restrict the population of interest (both cases and controls) to individuals reporting forest or forest-fringe exposure. Consider including both or one of the following: 1. A set of community controls matched at the village level. 2. Cases identified through active screening activities and a random selection of controls included in the active screening. Focus the questionnaire on forest-related exposures and defining activities (e.g., evening and early morning behaviors and travel patterns within the forest).
Cross border travel	In some settings, a higher proportion of cases are imported. Identifying specific patterns of human movement and reasons for travel associated with higher risk will be important for targeting screening procedures.	Restrict the population of interest to cross-border travelers. Consider including cases identified through active screening activities and a random selection of those included in the active screening as controls. Focus the questionnaire on cross-border travel patterns (e.g., specific destinations and travel routes), reasons for travel and defining high-risk behaviors in that context.

Appendix 4: Field Staff Responsibilities

Field Coordinator

- Ensure that field personnel are punctual and have a professional demeanor.
- Support the health facility staff to ensure that there is a secure place to store materials and equipment.
- Manage expenses.
- Ensure the availability of all survey materials.
- Based on supervision of recruitment and interviews at health facilities, provide feedback to the Interviewers and Nurses to improve procedures for data collection and fix problems identified by the team.
- Store samples and documents (consent forms, field notes, enumeration and other forms) in a safe, secure place.
- Ensure proper documentation of all survey activities, using the tablets, spreadsheets and forms.
- Review, tabulate, and reconcile questionnaires, forms and logs used in the field. Review errors with field staff.
- Write weekly progress reports used by the field team and PI to monitor recruitment.
- Conduct daily debriefings by phone or in person to assess the procedures for data collection, challenges, and how to improve data quality
- Conduct weekly meetings with the larger survey team (including investigators) to communicate and discuss progress and adjust the planning of the survey, as necessary.
- Supervise and monitor the work of the field teams, particularly early in data collection (Interviewers and Nurses).

Interviewers

- Ensure that all forms, DBS cards and other supplies are prepared for each day.
- Promptly assess eligibility and complete the eligibility screening form of any individual tested for malaria by the Nurse.

- Recruit participants for interviews and enter details into line listing.
- Assign a unique participant ID and ensure that the participant ID entered in the questionnaire matches the participant ID put on the malaria tests and blood samples.
- Conduct informed consent.
- Conduct interviews.
- Ensure that all questionnaires are completed and uploaded at the end of the day.
- Organize and store all malaria tests and blood samples collected by the Nurse, to be picked up by the Field Coordinator.
- Organize and store all study documentation (i.e., informed consent and line listings) for review and pick up by the Field Coordinator.
- Maintain data integrity (i.e., all data collected accurately represents the information provided by participants).
- Comply with guidelines for maintaining safety, data security, and participant confidentiality.
- Implement local safety procedures and report field incidents to the Field Coordinator immediately.
- Conduct daily inventory of all supplies, and communicate with the Field Coordinator when any supplies are low or need to be replenished.

One staff member may assume the role of both the Interviewer and Nurse if health facility burden allows.

Nurses

- Take blood samples from participants using venipuncture and finger-sticks.
- Create DBS for analysis.
- Coordinate with Interviewers to properly store and transport the samples.
- Provide treatment and referrals according to regular test results and national guidelines.

One staff member may assume the role of both the Interviewer and Nurse if health facility burden allows.

Appendix 5: Participant Identification Codes

In order to keep each participant's information confidential, we have created codes that will be used in place of the participant's names. The code is 6 digits long and correspond to a barcode/sticker that will be used on all forms associated with the participants (health facility notification log, informed consents, survey questionnaire, participant tracking forms), in addition to all the biological samples (slide, dried blood spot, RDT, whole blood). When you return your completed forms to the Field Coordinator, be sure to keep all those with the same code together and stapled. Each digit within the code has a significant meaning that corresponds to the study site, study component and a unique number corresponding to that individual.

The breakdown of the code is as follows:

Subdistrict code (1 digit)	Interview type (2 digits)	Unique ID (3 digits)

The first digit of the code is based on your study location, or subdistrict. If there are two study locations with the same initials or name, make sure that they are coded differently. An example of the digits for this code is as follows:

Subdistrict	Code
KG	1
LH	2
SA	3
RG	4

The second and third digits in the code are for the study component participant type that is being enrolled into the study. Depending on the study component, use one of the following codes:

Participant type	Code
Index case	01
Health facility control	02
Community control	03

Lastly, the final 3 digits are unique to each individual and will be between 001 and 999. Each participant is to have a different number so no two providers or patients will have the same final 2 digits. Here are some examples of codes you will write on each form used for each participant.

An index case in KG1 would have the following code:

1	01	001

Sub district code	Patient category	Participant unique ID
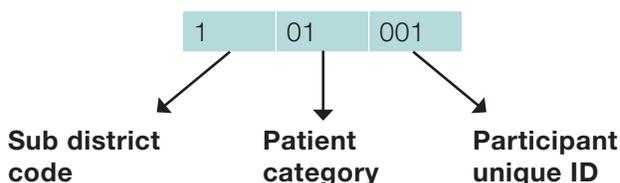
Using Barcodes

The barcodes should be pre-printed onto stickers for ease of use. Barcode sheets allowing at home printing (Avery 5428) include four barcode stickers for each participant. The three left columns contain single barcodes and the right column contains double barcodes. The double barcode will enable the sample to be cut in half so that two spots are available for analysis at each of several facilities. The use of these barcodes is as follows:

Column 1	Column 2	Column 3	Column 4
Case/ control line list	Informed Consent Form	RDT/blood slide	Dried Blood Spot (double bar code)

Note: the date and identifier will need to be hand written on the other copy of the informed consent for the patient to keep.

Appendix 6: Eligibility Screening Form

Participant ID (if applicable): _____

Instructions: Complete the entire screening form for every individual tested for malaria.

Only questions that are not in brackets should be made to the participant. Circle the answer to each criteria. If the person is eligible to participate, enter the person into the line list and continue with informed consent.

Date: __ __ / __ __ / __ __ __ __ (DD/MM/YYYY)			
Health Facility:			
Criteria		**Case**	**Control**
Must be 'YES' to be eligible	[RDT result]	Positive result YES / NO	Negative result YES / NO
Must be 'NO' to be eligible	[Clinical Diagnosis only?]	YES / NO	YES / NO
	Have you had a malaria diagnosis in the last four weeks?	YES / NO	YES / NO
	Have you taken malaria treatment in the last 14 days?	YES / NO	YES / NO
	[Monthly recruitment target met?]	Not applicable	YES / NO
Is the candidate eligible?	YES / NO		

If NO, why is candidate not eligible? [mark all that apply]

☐ Has an invalid test or diagnosed clinically?

☐ Has had a prior malaria diagnosis in preceding month?

☐ Has taken malaria prophylaxis or treatment in preceding 14 days?

☐ Is present at the clinic because he/she was accompanying someone with fever

☐ Is critically ill and is excluded from participation

☐ Is unable to communicate in understood language

☐ Other (specify): _____

Appendix 7: Health Facility Case Line List

Instructions: Enter all eligible cases into the line list and assign a participant ID to those who provide informed consent.

Date diag-nosed	Health facility	Individual details					Participant ID	Informed consent?	Interview?	DBS?
		Name	Age	Gender	Tel #	Occupation				
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
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								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐
								☐	☐	☐

Appendix 8: Health Facility Control Line List

Instructions: Enter all eligible controls into the line list and assign a participant ID to those who provide informed consent.

Date diag-nosed	Health facility	Individual details						Participant ID	Informed consent?	Interview?	DBS?
		Name	Age	Gender	Tel #	Occupation					
									☐	☐	☐
									☐	☐	☐
									☐	☐	☐
									☐	☐	☐
									☐	☐	☐
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									☐	☐	☐
									☐	☐	☐

Appendix 9: Monthly Control Recruitment Tracker

Instructions: Monthly control recruitment targets should be set based on the expected total number of cases in the study area and the number of controls needed for acceptable statistical power, divided by 12. The proportion of males/females recruited should match the case distribution. Age ranges should be restricted to 15 years and up if all cases are adults, or frequency matched based on the expected case distribution.

If the number of individuals tested for malaria every month far exceeds the number of expected controls (based on the formative review), use a skip pattern.

Month: _____ **Year:** _____ **Health Facility:** _____

Previous Month Rollover: Enter the number of individuals in each control category that could not be recruited in previous months. These individuals should be recruited before the current month recruitment begins.

Gender	Males		Females		Total
Age category	0–14	15+	0–14	15+	
Number to Rollover	1	2	0	0	3
Monthly Recruitment					

Current Month Recruitment: enter the number of individuals in each control category to be recruited at each health facility in the current month in the first row. A tally mark should be made for each participant recruited in this category. Recruitment for the month can cease after the target has been met.

Gender	Males		Females		Total
Age category	0–14	15+	0–14	15+	
Target Recruitment	5	10	1	2	18
Monthly Recruitment					

Appendix 10: Informed Consent Form

Name: _____

Study ID: _____

Malaria Elimination Risk Factor Assessment Tool (MERFAT)

Consent to participate in research

Study Title: Identification of risk factors for malaria in Bezi: a case-control study

My name is _____and I am working on a study with _____ (*program name/ organization*), funded by _____ (*name of funder*). This study will help to understand the causes of malaria in (*name of project area*) and gather information that will help to eliminate malaria here. You/your child are/is being asked to take part in the study because either you have been diagnosed with malaria or you have tested negative and can provide a comparison for those with malaria. If the patient is legally defined as a child (under 18 years of age), we also need to obtain consent from a parent or guardian.

Confidentiality and Consent

If you agree to participate in this study, I am going to take an additional fingerprick blood sample and ask some questions to understand your exposures to malaria, including where you live, your travel history and malaria knowledge and protection. The questions are not sensitive and your answers are completely confidential. You do not have to answer the questions if you do not want to, and you can finish this interview anytime. Participation or refusal to participate will not affect you or your child's medical care or access to public health services. However, your honesty in answering these questions will help us understand what behaviors and kinds of activities might lead to malaria in (*name of project area*). The information we put together will be useful for helping the malaria program to develop strategies to prevent and respond to malaria in this area.

We appreciate your help responding to this interview which will take about 45 minutes.

Consent

Do you agree to provide a blood sample to be used for current and future malaria testing? ☐ **Yes** ☐ **No**

Do you agree to participate in the questionnaire? ☐ **Yes** ☐ **No**

If you wish to participate, please sign or provide a thumb print below.

_____   _____

Date   Participant's signature/thumb print for consent

_____   _____

Date   Witness signature (if participant does not speak/read English) or Parent or Guardian signature if participant is under 18 years, if participant is over 10 the child's signature must also be obtained above.

_____   _____

Date   Interviewer's signature

Note: The Informed Consent form should be adapted to meet any organizational/institutional requirements.

Appendix 11: Guidance on Conducting Interviews with Participants

Successful interviewing is an art and should not be treated as a mechanical process. Each interview is a new source of information, so make it interesting and pleasant. Follow general guidelines below on how to build rapport with the participant and conduct a successful interview.

Building Rapport

The participant's first impression of you will influence their willingness to cooperate. Be friendly, respectful and smile as you introduce yourself. You will also be given an identification badge to wear at all times and a letter that states that you are working with the [name of institution or organization] on malaria surveillance.

Assure Confidentiality

If the participant is hesitant about responding or asks what the data will be used for, explain that the information you collect will remain confidential, their name will not be used for any purpose, and all information will be grouped together for statistical analysis and reports about malaria.

Never mention information from other interviews or show completed interview forms in front of a participant or anyone else.

Interview the Contact Alone

The presence of other people during an interview can prevent you from getting frank, honest answers. It is therefore very important that the individual interview be conducted privately and that all questions be answered by the participant.

Answer Questions Frankly

Before agreeing to be interviewed, the participant may ask you about the interview or why they were selected to be interviewed. Be direct and pleasant when you answer.

The participant may also be concerned about the time or length of the interview. If they ask, tell them that the interview usually takes about 30 to 60 minutes.

Participants who work in the forest or migrant workers may be concerned that you will ask them about illegal activities or testing for drug use. Explain to them that:

- Testing is for malaria, not for illegal drug use, or for other diseases
- They will not be asked about illegal activities. If the work they are engaging in is illegal, they will not be asked any of those details and they can choose not to provide any details at any time.
- Remind the participant that the interview is completely confidential and you will not be sharing any of the information with anyone outside of the surveillance team.

Participants may ask questions or want to talk further about the topics you bring up, such as indoor residual spraying or how to use a mosquito net. It is important not to interrupt the flow of the interview, so tell them that you will be happy to answer their questions or to talk further after the interview. After the interview is over, if you feel comfortable doing so, you may answer basic health or other questions to the best of your ability while informing the participant that you are not a nurse, doctor or expert on the topic. Give the participant the health information materials and refer them to local health staff for more information.

Maintain a Neutral Attitude

Interviewers should be sympathetic listeners and avoid giving the impression of having strong views on the subject under discussion. Neutrality is essential because some participants, trying to be polite, will say what they think the interviewer wants to hear.

If the participant gives an unclear answer, try to probe in a neutral way, asking questions such as the following:

- "Can you explain a little more?"
- "I did not quite hear you. Could you please tell me again?"
- "There is no hurry. Take a moment to think about it."

Never Suggest Answers

If a participant's answer is not relevant to a question, do not prompt them by saying something like "I suppose you mean that…Is that right?" In many cases, they will agree with your interpretation of their answer, even when that is not what they meant. Rather, you should probe in such a manner that the participant themselves come up with the relevant answer. You should never read out the list of coded answers to the respondents, even if they have trouble answering.

Do Not Force Participants to Answer Questions

If the participant is reluctant or unwilling to answer a question, explain once again that the same question is being asked of many people and that the answers will all be merged together. If the participant is still reluctant, select the "Refused to answer" option on the question and proceed as if nothing had happened. Remember, the participant cannot and should not be forced to give an answer.

Phrase Questions Carefully

Avoid questions that can be answered by a simple yes or no. For example, questions such as "Please tell me about malaria prevention?" are better than "Do you know about malaria prevention?"

Use Probing Techniques

Encourage participants to detail the basis for their conclusions and recommendations. For example, an informant's comment, such as "The malaria program has really changed things around here," can be probed for more details, such as "What changes have you noticed?" "Who seems to have benefitted most?" "Can you give me some specific examples?"

Appendix 12: Sample Collection and Storage

Background

Malaria is caused by parasites from the *Plasmodium* genus. There are 5 *Plasmodium* species that are known to cause malaria in humans. *Plasmodium falciparum* is the most predominant species, although *P. vivax* cases have also been recorded in many settings. These parasites infect human red blood cells and live in human blood. Malaria-causing parasites can therefore be isolated from blood samples.

Samples are normally collected in the field or at a health facility, stored within a specified range of temperature, and then transported to a well-equipped laboratory for the screening of target organisms. If blood samples are not stored properly, the malaria parasites may not be able to be identified. It is therefore important that the blood samples are collected and stored properly so that malaria parasites can be reliably identified.

Key Terminology

Sampling: The process of obtaining biological specimens such as blood, sweat, urine, or skin tissue by venipuncture or use of swabs from a source individual within a defined population.

Sample/specimen: This includes whole blood, urine, cheek tissue, feces, plasma, skin and hair from human or animals. There are also instances where water, air or soil maybe termed "samples" if they are being analyzed/screened for the presence of a particular agent.

Coding: A method of assigning unique identifiers, comprised of characters so that the identity of the person from whom the sample was collected can be concealed while at the same time linking the person's data to their location of residence, test results, treatment etc.

Rapid Diagnostic Testing (RDT) cassette: A cassette that detects parasite antigens or antibodies depending on the design. Results are rapidly available and can be read through a screen by the presence or absence of a line.

Dry Blood Spot (DBS): A folded paper containing a filter paper with equally sized, stamped rings on which finger-prick blood is collected on the circles. The blood is allowed to dry and the paper is subsequently folded to close.

Storage and preservation: The manner by which samples are bottled or placed in a bag, to ensure they are not subjected to damage due to the surrounding environmental conditions (e.g., temperature, moisture, pH, chemicals). Samples should be kept away from moisture and direct sunlight.

Sample Collection

In most countries, blood samples will be drawn from patients by a qualified health practitioner, who may be an Enrolled/Registered Nurse, Medical Laboratory Technologist or Medical Doctor. This may vary in different contexts but should always align with national guidelines. A malaria test using microscopy or RDT will have already been conducted to diagnose all eligible participants for MERFAT. The Interviewer will need to ask the Nurse to conduct a DBS for all consenting MERFAT participants.

Sample Coding

Every blood sample should be linked to the correct person, so that the malaria test results can be matched to the completed questionnaire. This is done using the unique Participant ID codes that are printed on the barcode labels. After a person has gone through the informed consent process, they are assigned a unique Participant ID that is used across the barcode labels.

Sample barcodes (top) and placement of barcode stickers on DBS and RDT (below).
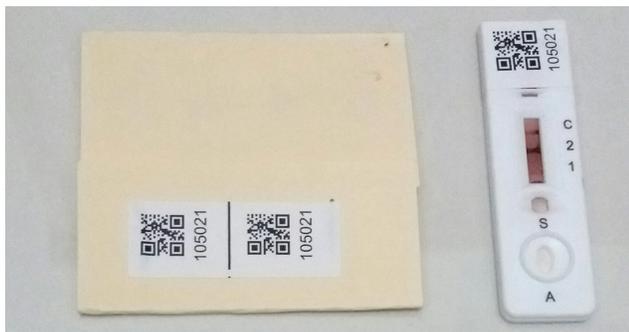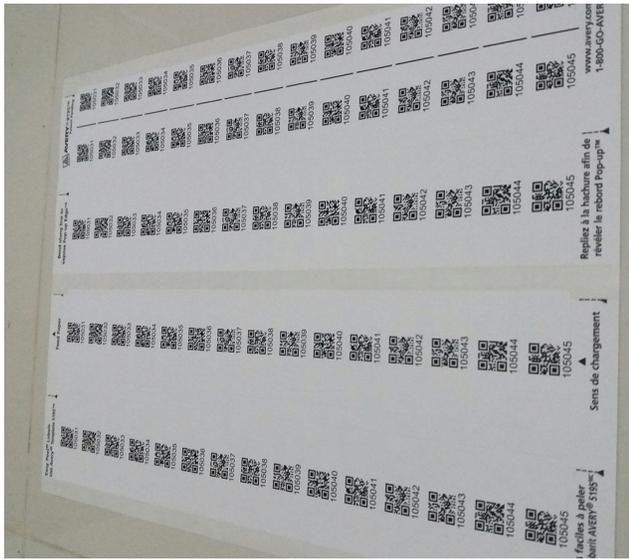
Three labels have been printed, which should be put on the 1) informed consent form, 2) slide or RDT and 3) DBS. The label with two barcodes should be put on the DBS, as shown on the pictures above.

Example: If Patient X has consented to participate in MERFAT, then the informed consent form, the slide or RDT, and the DBS from Patient X should all have the same barcode label with the same Participant ID.

- **Slide/RDT**: If the person has already been tested for malaria and is included in MERFAT as a case or a control, they will already have a slide or RDT. The Interviewer must ask for this from the Nurse and then label it with the barcode label.

- **DBS**: DBS cards will need to be labeled prior to giving them to the Nurse so that they may take the blood sample. It is best to request this sample at the end of the interview so that the Interviewer can move on to interviewing the next person.

Sample Handling

Although the Nurses will be responsible for conducting the malaria test and collecting the DBS, Interviewers will need to handle the samples in order to dry them and store them. The following protocol should be followed.

1. Samples should be left to dry in an area that has good ventilation and is protected from the wind, insects or people touching them. Samples should not be exposed to direct sun. The sample should be left to dry until the blood spot appears brown (approximately 10 minutes).

2. Once they are dry, the flap should be folded down to cover the blood spots

The white filter paper should never be touched. Only the yellow cardstock cover can be touched. It is mandatory to wear gloves when handling samples for coding and examination purposes.

It is good practice to always disinfect your hands after handling samples, even if you have not come in direct contact with it.

Barcode placement on DBS

Sample Organization and Storage

Once the DBS is dry, it should be neatly stacked into trays together with the slide or RDT for organization and storage. Ideally, stacks of filter paper cards should remain stable and organized, and not shift within a box.

Samples should be sorted in order of the Participant ID, with the slide or RDT and DBS with the same code stored next to each other.

The tray of samples should be placed in a zip lock bag containing a humidity sponge/desiccant. Two

trays should be fit into a zip lock bag. Before the bag is sealed, excess air should be squeezed out. A permanent marker pen should be used to clearly label the zip lock bags with the date of sample collection and the health facility from which the samples were collected. Immediately store in the cool box to preserve the sample.