**Protocol Template: Clinical Performance and Usability Evaluation of Malaria Rapid Diagnostic Tests (RDTs)**

# Preface

Remove this **Preface** before using this clinical study protocol.

This document is a template protocol for a clinical performance and usability evaluation of one or more malaria rapid diagnostic tests (RDTs). The goal of this template is to assist researchers, manufacturers, and investigators in developing a comprehensive clinical study protocol for studies of this nature and in considering key assumptions and elements of study design, planning, and execution. The sections described in this template include content required for a clinical performance study protocol as per ISO 20916:2019.

## How to use this template

This template contains two types of text: explanatory text and example text.

- **Explanatory text** is indicated at the beginning of each section in *italics with a pink background* and should be deleted. The purpose of this text is to describe the content that should be included in the relevant section and to provide instructions and guidance related to key considerations for protocol design and development.
- **Example text** is included to facilitate protocol development. It can either be incorporated into the protocol as written; modified to align with the product under evaluation, the study design, and the study procedures/conduct; or deleted. Example text is indicated in regular font. Within example text, a need for insertion of study- or context-specific information is indicated with [bracketed text with a gray background].

## Disclaimer

This protocol template is provided by PATH for informational and educational purposes only. PATH does not assume any responsibility or liability for studies that adopt or modify this template. Investigators and sponsors are solely responsible for ensuring that any clinical study using this template complies with applicable laws, regulations, institutional policies, and ethical standards. This template is not intended to replace regulatory guidance or formal clinical development planning. Protocols intended to generate data for regulatory submissions must be developed within a comprehensive clinical plan that is aligned with applicable regulatory requirements.

## Resources

Additional resources that may be supportive in the development of a clinical study protocol of this nature include the following:

- World Health Organization (WHO). *Technical Specifications Series for Submission to WHO Prequalification – Diagnostic Assessment. TSS-3: Malaria Rapid Diagnostic Tests*. WHO; 2017. Licence: CC BY-NC-SA 3.0 IGO. https://iris.who.int/bitstream/handle/10665/255038/9789241512275-eng.pdf?sequence=1
- World Health Organization (WHO). *Technical Guidance Series for WHO Prequalification – Diagnostic Assessment: Principles of Performance Studies*. WHO; 2017. Licence: CC BY-NC-SA 3.0 IGO. https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf?sequence=1
- International Organization for Standardization (ISO). ISO 20916:2019. *In Vitro Diagnostic Medical Devices — Clinical Performance Studies Using Specimens From Human Subjects — Good Study Practice*. ISO; 2019.
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). *ICH Harmonised Guideline: Guideline for Good Clinical Practice: E6(R3)*. ICH; 2025. https://database.ich.org/sites/default/files/ICH_E6%28R3%29_Step4_FinalGuideline_2025_0106.pdf

## Suggested citation

PATH. *Protocol Template: Clinical Performance and Usability Evaluation of Malaria Rapid Diagnostic Tests*. Version 1.0. PATH; 2025.

## Funding

This work was supported by a grant from the Gates Foundation (INV-071824).

## Protocol template version history

| Version | Version date | Description |
|---|---|---|
| 1.0 | June 25, 2025 | Initial version |

# Title page

## Protocol title: Clinical performance and usability evaluation of the [name(s) of investigational product(s)] malaria rapid diagnostic test[s]

| | |
|---|---|
| **Protocol short title** | |
| **Protocol number** | |
| **Sponsor** | |
| **Protocol version number** | |
| **Date of protocol version** | |
| **Principal Investigator** | |
| **Location of research** | |
| **Reviewing ethics committee(s)** | [Institution name], [address], [contact information] |

*Reminder: Track versions during protocol development and study implementation, using best practices for document management and version control.*

### Summary of changes from previous versions

| Version | Version date | Affected section(s) | Reason for new version/description of changes |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

# Contents

*Reminder: Select Microsoft Word's "Update entire table" option to align this table of contents with the final protocol.*

# Abbreviations

| | |
|---|---|
| CRF | Case Report Form |
| GCP | Good Clinical Practice |
| HRP2/*hrp2* | histidine-rich protein 2 (protein)/*histidine-rich protein 2* (gene) |
| HRP3/*hrp3* | histidine-rich protein 3 (protein)/*histidine-rich protein 3* (gene) |
| ICH | International Conference for Harmonisation |
| IRB | institutional review board |
| IVD | in vitro diagnostic |
| LDH | lactate dehydrogenase |
| LOD | limit of detection |
| MOP | manual of procedures |
| NPV | negative predictive value |
| PCR | polymerase chain reaction |
| pLDH | *Plasmodium* lactate dehydrogenase |
| PPV | positive predictive value |
| qPCR | quantitative polymerase chain reaction |
| RDT | rapid diagnostic test |
| SOP | standard operating procedure |
| TSS | Technical Specification Series |
| WHO | World Health Organization |

# Study personnel and institutions

| | |
|---|---|
| **Sponsor** | [Institution name], [address] |
| **Funder** | [Institution name], [address] |
| **Manufacturer of investigational product[s]** | [Institution name], [address] |

**Principal Investigator**

[Name], [professional position], [affiliation], [contact information]

**Co-Investigators**

[Name], [professional position], [affiliation], [contact information]

[Name], [professional position], [affiliation], [contact information]

[Name], [professional position], [affiliation], [contact information]

[Name], [professional position], [affiliation], [contact information]

# Statement of compliance

*Include a statement that the study will be conducted in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), ethical principles (e.g., the Declaration of Helsinki), and all applicable national and local regulatory requirements. The statement should also clarify that the study will not begin until the required approvals have been obtained from the responsible ethics committees and/or regulatory authorities, as applicable. An example of such a statement is included below.*

The study will be conducted in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable state, local and federal regulatory requirements. The Principal Investigator will ensure that no deviation from, or changes to, the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board[s] (IRB), except where necessary to eliminate an immediate hazard(s) to the study participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP training.

The protocol, informed consent form[s], recruitment materials, and all participant materials will be submitted to the IRB[s] for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB[s] before the changes are implemented to the study. All changes to the consent form[s] will be IRB approved.

# 1. Protocol synopsis

| | |
|---|---|
| **Protocol title** | Clinical performance and usability evaluation of the [name(s) of investigational product(s)] malaria rapid diagnostic test[s] |
| **Summary** | *Include a brief summary of the study design and procedures. Example text is included below, but this should be updated to align with the testing workflow for the study. Text in this section should also align with the main body of the protocol.*<br><br>Prospective, noninterventional, cross-sectional diagnostic accuracy and usability study with [number] patient participants and [number] lay provider/health care worker participants. Febrile individuals suspected of malaria will be recruited at clinics. Following enrollment, study staff will collect capillary blood samples and conduct the standard-of-care malaria test and [list the investigational test(s)]. All clinical management of study participants will follow the standard of care in [country] and will be based on the standard-of-care test result. Venous blood will be collected and transferred cold to the laboratory where research-grade microscopy slides will be prepared, the reference polymerase chain reaction (PCR) assay will be run, and investigational and comparator RDTs will be performed. Specimens will be aliquoted in the laboratory and stored frozen for confirmatory testing. Confirmatory testing may include typing and sequencing of *Plasmodium* genes and antigens of interest, including but not limited to histidine-rich protein 2 (HRP2), histidine-rich protein 3 (HRP3), and *Plasmodium* lactate dehydrogenase (pLDH).<br><br>The lay provider/health care worker participants in the usability study will include intended users of malaria RDTs. They will be surveyed to assess the usability of the investigational test[s] through a questionnaire to assess label and packaging comprehension as well as results interpretation. |
| **Objectives** | *List the study's primary and secondary study objectives. These should be the same as those listed in the body of the protocol. Examples are provided below, and specific guidance on setting primary and secondary objectives and endpoints is described in section 3 of this template.*<br><br>**Primary**<br>1.1 To assess the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)—altogether referred to hereafter as "diagnostic accuracy"—of the [investigational test] in intended use settings for detecting [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.<br>1.2 To assess the diagnostic accuracy of the [second investigational test, if applicable] in intended use settings for detecting [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.<br><br>**Secondary**<br>2.1 To assess the diagnostic accuracy of the comparator tests in intended use settings for detecting [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.<br>2.2 To determine the frequency of *P. falciparum* infections containing histidine-rich protein 2 (*hrp2*) and/or histidine-rich protein 3 (*hrp3*) gene mutations. |

| | |
|---|---|
| | 2.3 To assess the performance of the investigational and comparator tests on *P. falciparum* infections with *hrp2* and/or *hrp3* mutations.<br>2.4 To assess comprehension of the [investigational product(s)] packaging and labeling among intended users (trained lay providers and health care workers).<br>2.5 To assess the ability of intended users (trained lay providers and health care workers) to read and interpret the [investigational product(s)] test results. |
| **Endpoints** | *List the study's primary and secondary study endpoints. These should be the same as the endpoints listed in the body of the protocol. Each endpoint should correspond to one or more study objectives as listed above. Examples are provided below, and specific guidance on setting primary and secondary objectives and endpoints is described in section 3 of this template.*<br><br>**Primary**<br>1.1 Estimates of diagnostic accuracy characteristics (sensitivity, specificity, NPV, PPV), with 95% confidence intervals, of the [investigational test] for the detection of [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.<br>1.2 Estimates of diagnostic accuracy characteristics (sensitivity, specificity, NPV, PPV), with 95% confidence intervals, of the [second investigational test, if applicable] for the detection of [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.<br><br>**Secondary**<br>2.1 Estimates of diagnostic accuracy characteristics (sensitivity, specificity, NPV, PPV), with 95% confidence intervals, of the comparator tests in intended use settings for detecting [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.<br>2.2 Frequency of *P. falciparum* infections containing *hrp2* and/or *hrp3* gene deletions.<br>2.3 Estimates of sensitivity, with 95% confidence intervals, of the investigational and comparator tests for the detection of *P. falciparum* with *hrp2* and/or *hrp3* deletions.<br>2.4 Percentage of intended users who can accurately comprehend key messaging included in the [investigational product(s)] packaging and labels.<br>2.5 Percentage of intended users who can accurately interpret the [investigational product(s)] result outputs. |
| **Study population** | *Specify the sample size, geographic location, and relevant demographic/health status characteristics of the study population groups.*<br><br>For the diagnostic accuracy study, the sample size is set at [number] patients, aged 2 years and older, presenting at the recruiting facility in [location] with symptoms suggestive of malaria. Based on expected prevalence at the site[s], this sample is expected to include approximately [number] *P. falciparum* infections, [number] *P. vivax* infections, and [number] malaria negative by PCR.<br><br>The sample size for the usability study is [number] lay providers/health care workers. |
| **Eligibility criteria** | **Inclusion criteria: patient participants (diagnostic accuracy study)**<br>• Aged 2 years or older. |

| | |
|---|---|
| | • Presenting at the study site with a fever or history of fever during the preceding 48 hours.<br>• Freely agreeing to participate by providing informed consent (and assent, as applicable).<br><br>**Exclusion criteria: patient participants (diagnostic accuracy study)**<br>• Serious illness, defined as illness requiring referral or hospitalization as determined by the responsible health care provider.<br><br>**Inclusion criteria: lay providers/health care workers (usability study)**<br>• Aged 18 years or older.<br>• Provides malaria case management.<br>• Considered an intended user of malaria RDTs (lay provider or health care worker).<br>• Freely agreeing to participate by providing informed consent.<br><br>**Exclusion criteria: lay providers/health care workers (usability study)**<br>None |
| **Study site(s)** | *Provide a brief description of planned facilities/participating sites that will enroll participants. Indicate the number of recruiting sites.*<br><br>This study will be conducted at [list study site(s) and location of research activities]. |
| **Duration of study** | *Provide the estimated time (in months) from when the study opens to enrollment until completion of data analyses.* |
| **Study products** | **denotes optional tests*<br><br>Investigational product[s]:<br>• [Investigational product 1].<br>• [Investigational product 2, if applicable].*<br><br>Comparator tests:<br>• [Standard-of-care malaria test—either RDT or facility-based microscopy].<br>• [Comparator RDT 1].<br>• [Comparator RDT 2, if applicable].*<br>• [Additional comparator RDTs, as applicable].*<br>• Research-grade light microscopy.<br><br>Reference test:<br>• PCR assay *(note: ideally, real-time polymerase chain reaction [PCR] for quantification and species identification)*.<br><br>Confirmatory test(s):<br>• Assay for *hrp2*/*hrp3* gene deletion characterization in *P. falciparum* infections.*<br>• Malaria antigen quantification assay.* |

# 2. Background and rationale

*Provide relevant background information and describe the rationale for conducting the study. This section should describe the problem/question that the study aims to address; applicable clinical, epidemiological, or public health background or context for the study relevant to the disease or condition of interest (in this case, malaria) and population; the current standard-of-care tests and their limitations; and the relevant available evidence (including preclinical or analytical studies) related to the investigational product(s). The claims and intended performance of the rapid diagnostic test(s) (RDT[s]) under evaluation in the study should be described, and the study design should be appropriately justified, including the need for prospectively collected specimens. Relevant literature should be reviewed and cited, with citations listed in section 14.*

*Sample text is provided below to describe the need for development and clinical evaluation of more sensitive malaria RDTs.*

Early and accurate diagnosis of malaria is essential both for effective management of the disease and for strong surveillance. For over a century, microscopy has enabled the direct visualization of malaria parasites, and it continues to be widely used today. However, the quality of microscopy is highly variable and dependent on factors such as operator proficiency, quality of materials, and methods.[1] In the 1990s, lateral flow immunochromatographic rapid diagnostic tests (RDTs) for malaria were first introduced. The simplicity, low cost, minimal infrastructure requirements, and rapid time to results of these tests make them particularly well suited for malaria case management and enable point-of-care confirmation of infection prior to treatment, even in remote settings. Following the World Health Organization's (WHO's) 2010 policy recommendation that antimalarial treatment be administered following confirmation of infection by either microscopy or RDT, the use of RDTs expanded significantly. These tests have now been widely accepted in endemic settings.[2,3]

Malaria RDTs detect specific antigens produced by *Plasmodium* parasites in human blood. These antigens bind to dye-labeled antibodies contained in a nitrocellulose test strip, creating a visible band.[4] Most currently available malaria RDTs rely on the detection of the antigens histidine-rich protein 2 (HRP2 and/or *Plasmodium*-specific lactate dehydrogenase (LDH).[5] HRP2 is a *P. falciparum*–specific malarial protein that is excreted in high abundance by the parasite throughout the different stages of its life cycle. In contrast, LDH is commonly expressed by all *Plasmodium* species. The vast majority of RDTs that are used for detecting *P. falciparum* target HRP2 due to its species specificity, heat stability, and higher documented clinical performance as compared to LDH-based RDTs.[6,7,8] The use of malaria RDTs for the detection of *P. vivax*, the most widespread species outside of sub-Saharan Africa, has remained limited in many settings due to poor LDH sensitivity, resulting in continued reliance on microscopy.[9]

However, HRP2-based malaria RDTs have several notable limitations. The HRP2 antigen can persist in blood for more than 3 weeks after parasite clearance and can therefore yield false-positive results among patients who have recently received treatment.[5] Most importantly, there have been increasing reports of parasites with deleted *hrp2/hrp3* genes following initial observations from the Amazon region starting in 2010.[10] The gradual spread of *hrp2*-deleted mutants in several endemic countries in South America, Asia, and Africa has exerted a substantial potential impact on the utility of HRP2-based tests for case management in these settings.[11] For these reasons, development of RDTs with improved analytical sensitivities for pLDH is currently considered to be of high priority. Due to the documented lower sensitivity of LDH-based RDTs for *P. falciparum* in comparison to HRP2-based RDTs in the absence of

*hrp2/hrp3* deletions, WHO currently recommends switching to LDH-based RDTs when there is a confirmed significant prevalence (>5%) of false-negative RDT results arising from *hrp2/hrp3* deletions.[12]

To address these challenges, [manufacturer] has developed [describe investigational product(s), what antigen(s) it/they detect, and available evidence supporting their improved performance over current tests].

The aim of this study is to evaluate the clinical performance of [investigational product(s)] for the detection of [*Plasmodium* species] in a malaria-endemic intended use setting. The data collected in this study will be used to evaluate the product[s]' intended use and performance claims and will [or may] be included as part of regulatory dossiers. In addition, the study will generate valuable data on the performance of these test[s] in comparison to that of current standard-of-care products [and other comparator test(s), as applicable], which will enable informed decision-making regarding recommendation of new tools for malaria detection.

# 3. Objectives and endpoints

*Clearly stated objectives and endpoints are a critical component of study design and should articulate the goals/purpose of the study and inform the analysis. Careful consideration should be given to the amount and type of data needed to support the study's objectives.*

- *An **objective** is defined as the purpose for performing the study in terms of the specific scientific question to be answered. Objectives should be described as a statement of purpose (e.g., "to assess," "to evaluate," etc.). Each objective should correspond to one or more endpoints.*
- *An **endpoint** is a specific measurement that corresponds to a specific objective. Endpoints are also sometimes referred to as "outcomes."*

*The **primary objective**(s) is/are the main goal(s) of the study and should reflect the most important research question. Additional objectives and endpoints are secondary. The sample size calculation should be based on the primary endpoint(s). The statistical power of analysis involving a secondary objective is calculated based on the sample size for the primary objective.*[i]

*For a malaria RDT clinical performance evaluation, objectives and endpoints should be informed by several factors, including: (1) the species of malaria and the antigen(s) targeted by the investigational product(s), (2) the prevalence of malaria at the study site(s) for relevant species, and (3) the sample types being collected in the study.*

*For example, if the study aims to evaluate the clinical performance of an RDT that has two test lines, one for P. falciparum and one for P. vivax, but the study is being conducted in an area of low P. vivax prevalence, then primary objectives might relate to the test's performance for P. falciparum detection, whereas P. vivax–related performance analyses may be secondary.*

***Note:** The objectives and endpoints described in this section should align with those listed in the protocol synopsis in section 1 of this template.*

[i] The Pennsylvania State University's Department of Statistics. Lesson 5: Objectives and endpoints. STAT 509: Design and Analysis of Clinical Trials. Accessed May 16, 2025. https://online.stat.psu.edu/stat509/lesson/5

# 3.1 Objectives

## 3.1.1 Primary objectives

1.1 To assess the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)—altogether referred to hereafter as "diagnostic accuracy"—of the [investigational test] in intended use settings for detecting [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.

1.2 To assess the diagnostic accuracy of the [second investigational test, if applicable] in intended use settings for detecting [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.

## 3.1.2 Secondary objectives

2.1 To assess the diagnostic accuracy of the comparator tests in intended use settings for detecting [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.

2.2 To determine the frequency of *P. falciparum* infections containing histidine-rich protein 2 (*hrp2)* and/or histidine-rich protein (*hrp3)* gene mutations.

2.3 To assess the performance of the investigational and comparator tests on *P. falciparum* infections with *hrp2* and/or *hrp3* mutations.

2.4 To assess the comprehension of the [investigational product(s)] packaging and labeling among intended users (trained lay providers and health care workers).

2.5 To assess the ability of intended users (trained lay providers and health care workers) to read and interpret the [investigational product(s)] test results.

# 3.2 Endpoints

## 3.2.1 Primary endpoints

1.1 Estimates of diagnostic accuracy characteristics (sensitivity, specificity, NPV, PPV), with 95% confidence intervals, of the [investigational test] for the detection of [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.

1.2 Estimates of diagnostic accuracy characteristics (sensitivity, specificity, NPV, PPV), with 95% confidence intervals, of the [second investigational test, if applicable] for the detection of [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.

## 3.2.2 Secondary endpoints

2.1 Estimates of diagnostic accuracy characteristics (sensitivity, specificity, NPV, PPV), with 95% confidence intervals, of the comparator tests in intended use settings for detecting [*Plasmodium* species] infections in capillary and venous whole blood samples collected prospectively from patients with symptoms suggestive of malaria.

2.2 Frequency of *P. falciparum* infections containing *hrp2* and/or *hrp3* gene deletions.

2.3 Estimates of sensitivity, with 95% confidence intervals, of the investigational and comparator tests for the detection of *P. falciparum* with *hrp2* and/or *hrp3* deletions.

2.4 Percentage of intended users who can accurately comprehend key messaging included in the [investigational product(s)] packaging and labels.

2.5 Percentage of intended users who can accurately interpret the [investigational product(s)] result outputs.

# 4. Study design

## 4.1 Overall design

*Provide a summary of the study design and procedures. Example text is included below for a cross-sectional, noninterventional diagnostic accuracy study that recruits symptomatic patients in a facility setting and tests all enrolled participants with the same investigational, comparator, and reference tests through a paired design. This text should be modified to align with the testing workflow for the study and the specific investigational, comparator, and reference tests used. The description of the study design in this section should be consistent with the protocol synopsis in section 1 of this template.*

*Some additional considerations for study design include the following.*

- ***Capillary sample collection:***
    - ***Order of testing:*** *The standard-of-care malaria test should be prioritized and run first. Care should be taken to ensure that study tests do not interfere with patients' routine testing for malaria. Additional capillary blood drops can be used for investigational tests.*
    - ***Number of tests:*** *Efforts should be made to ensure that the number of tests run using capillary samples is reasonable, feasible within normal limits, and appropriately justified. Large numbers of finger pricks will increase participant discomfort and may increase the likelihood of withdrawals. It is recommended to limit capillary testing to necessary tests. Venous blood can be used for repeat testing with the investigational product(s) as well as comparator tests, with equivalence established between capillary and venous specimens.*
- ***Microscopy:*** *Even if microscopy is the standard-of-care test for malaria at the study site (with slides presumably prepared from capillary finger-stick samples), it is still advisable to conduct study-specific research-grade microscopy*[ii] *with slides prepared from venous blood. There is a high degree of variability in clinical microscopy practices globally. Resources to support the conduct of research-grade microscopy are described in section 8.2.1.*
- ***Comparator RDTs:*** *It is recommended to select a comparator RDT that is either currently used at the recruiting facility as the current standard of care or is World Health Organization (WHO) prequalified*

[ii] World Health Organization (WHO), United Nations Children's Fund/United Nations Development Programme/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). *Microscopy for the Detection, Identification and Quantification of Malaria Parasites on Stained Thick and Thin Blood Films in Research Settings (Version 1.0): Procedure: Methods Manual*. WHO on behalf of TDR; 2015. https://iris.who.int/handle/10665/163782

*and has a test line for histidine-rich protein 2 (HRP2). The list of WHO-prequalified products is available at https://extranet.who.int/prequal/vitro-diagnostics/prequalified-vitro-diagnostics.*

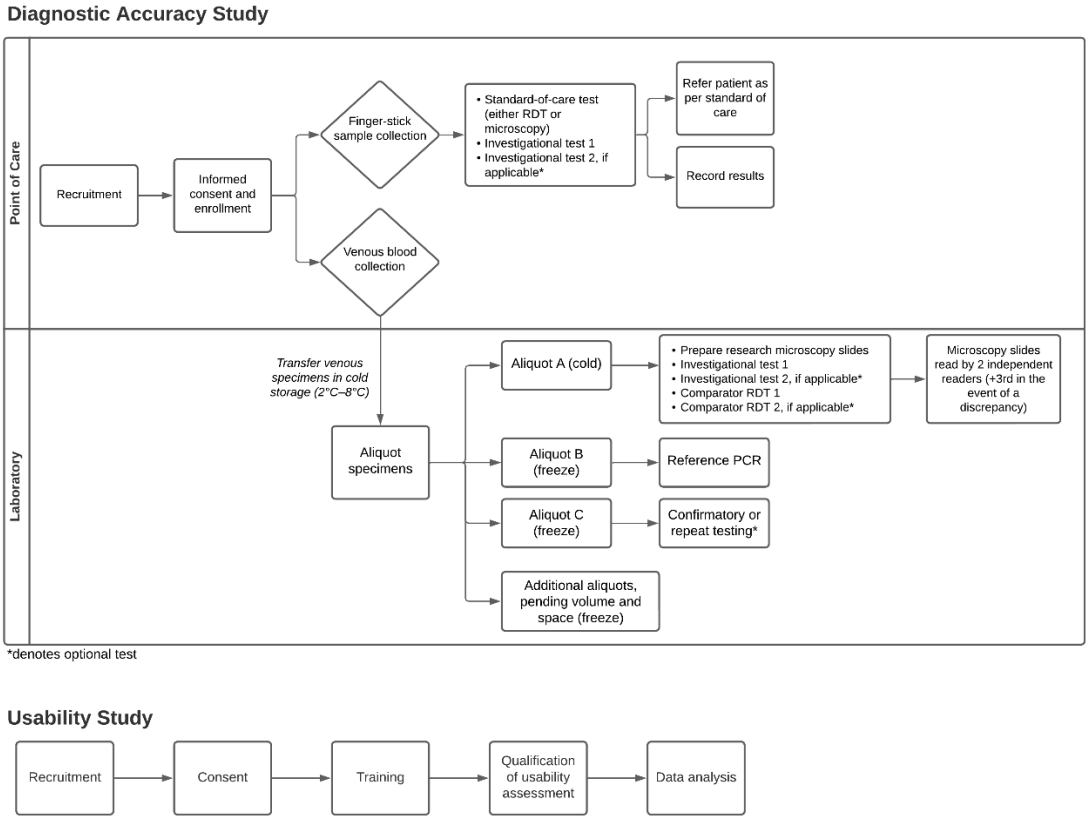
*The template workflow diagram shown as Figure 1 is available to be exported and copied for editing through Lucidchart at https://lucid.app/lucidchart/70488580-cffc-4913-b004-b493a024cccc/view.*

This is a prospective, noninterventional, cross-sectional diagnostic accuracy and usability study with [number] patient participants and [number] lay provider/health care worker participants. Symptomatic patients suspected of malaria will be recruited at clinics. Following enrollment, study staff will collect capillary blood samples and conduct the standard-of-care malaria test and [list the investigational test(s)]. All clinical management of study participants will follow the standard of care in [country] and will be based on the standard-of-care test result. Venous blood will be collected and transferred cold to the laboratory, where research-grade microscopy slides will be prepared, the reference PCR assay will be run, and investigational and comparator RDTs will be performed. Specimens will be aliquoted in the laboratory within [number] hours of collection and stored frozen for confirmatory or future testing. Confirmatory testing may include typing and sequencing of *Plasmodium* genes and antigens of interest, including but not limited to HRP2, HRP3, and pLDH.

The lay provider/health care worker participants in the usability study will include intended users of malaria RDTs. They will be surveyed to assess the usability of the investigational test[s] through a questionnaire to assess label and packaging comprehension as well as results interpretation.

An overview of the study design is shown in Figure 1 below, by component.

**Figure 1. Study design overview, by component.**



Note: PCR, polymerase chain reaction; RDT, rapid diagnostic test.

## 4.2 Scientific rationale for study design

*The scientific rationale for the study design should be justified, and measures taken to avoid bias should also be noted.*

*This template protocol describes a **cross-sectional study** of a consecutive series of prospectively enrolled symptomatic individuals presenting at clinics with symptoms of malaria. Diagnostic accuracy is evaluated through a paired (within subjects) design, where all subjects undergo testing with both the investigational and reference tests. This design will provide a representative spectrum of patients, including those with and without malaria, that aligns with the intended use of malaria RDTs; this is preferred over a case-control design as it avoids spectrum bias. The sample text provided below aligns with this study design.*

*However, investigators may wish to explore alternative designs (e.g., two-gate or case-control designs) that enrich the study population for specific types of less common samples (e.g., suspected hrp2/hrp3 deletions or certain species) based on the use of a screening test(s).*[iii] *Such designs should be carefully considered to minimize bias in investigational test result interpretation and performance analysis.*

Considering the minimally invasive nature of the malaria RDT[s] under evaluation, a paired (within subjects) study design is used because it allows for the use of different investigational, comparator, and reference tests from samples collected from the same patient and thereby enables the tests under investigation to be directly compared to the reference method. A prospective cross-sectional design will be used to avoid any bias in estimates of clinical performance characteristics.

## 4.3 End of study definition

The end of the diagnostic accuracy study is defined as the date when the last specimen from the last participant is tested on all testing platforms (including repeat testing if necessary) and all the results are reported.

## 4.4 Study site

*Describe the location of the study and its epidemiological characteristics relevant to malaria.*

This study will take place in [city], [country], at [name of site(s)]. In [year], [describe malaria burden, including estimated prevalence of predominant species and any reports of cases of *P. falciparum hrp2/hrp3* gene deletions, citing relevant literature].

## 4.5 Standard of care

*Describe the standard practices for malaria testing and case management at the study site. It is essential that all participants in the study receive the results of a valid, approved test to inform their treatment for malaria in a timely manner. Note that this template protocol describes a noninterventional study; as such, we assume that no changes to participants' routine care for malaria will result from their participation in*

[iii] Rutjes AWS, Reitsma JB, Vandenbroucke JP, Glas AS, Bossuyt PMM. Case-control and two-gate designs in diagnostic accuracy studies. *Clinical Chemistry*. 2005;51(8):1335–1341. doi:10.1373/clinchem.2005.048595

*the study. Under this scenario, all individuals, regardless of their participation in the study, should receive the standard-of-care test for malaria that will inform their treatment as per national guidelines.* ***However****, depending on the final study workflow and the regulatory status(es) of the included investigational and comparator products, the investigators should assess whether or not any study test result would need to be returned to participants/their providers to inform clinical care, as well as the risks and benefits to participants associated with doing so.*

*Additionally, if the standard-of-care test at the study site is an RDT, investigators should consider what could happen if stocks of RDTs are not available at any point during study implementation.*

As part of standard malaria case management at [site], suspected malaria cases are confirmed with [specify which: microscopy using a thick blood slide prepared with capillary blood and read by trained microscopists at the facility/RDT—specify name, manufacturer, and catalog number]. [Describe treatment algorithm, citing available national treatment guidelines].

# 5. Research participants

*In the following sections, the participant population(s) included in the study should be described. The included population(s) should be clinically relevant to the claim under evaluation.*

*Because this protocol template is for a cross-sectional study of symptomatic individuals presenting at clinics, the sample inclusion and exclusion criteria described below are fairly broad.*

## 5.1 Characteristics of research participants

### 5.1.1 Diagnostic accuracy study

Participants in the study will be symptomatic patients aged 2 years and older who are seeking care at [site] with a fever or history of fever within the preceding 48 hours.

### 5.1.2 Usability study

Participants in the usability study will be adult lay providers/health care workers over the age of 18 years who are responsible for malaria case management and testing as part of their roles. They will be representative of intended end users of malaria RDTs in [study location].

## 5.2 Inclusion criteria

*Inclusion criteria define the population under evaluation. Potential participants must fulfill these criteria in order to be enrolled into the study. The inclusion criteria listed below should align with those presented in the protocol synopsis in section 1.*

### 5.2.1 Diagnostic accuracy study

- Aged 2 years or older.
- Presenting at the study site with a fever or history of fever during the preceding 48 hours.
- Freely agreeing to participate by providing informed consent (and assent, as applicable).

### 5.2.2 Usability study

- Aged 18 years or older.
- Provides malaria case management.
- Considered an intended user of malaria RDTs (lay provider or health care worker).
- Freely agreeing to participate by providing informed consent.

## 5.3 Exclusion criteria

*Exclusion criteria are characteristics that make an individual ineligible for study participation. If specific populations are excluded, clear and compelling rationale and justification should be provided. The exclusion criteria listed below should align with those presented in the protocol synopsis in section 1.*

### 5.3.1 Diagnostic accuracy study

- Serious illness, defined as illness requiring referral or hospitalization as determined by the responsible health care provider.

### 5.3.2 Usability study

None.

## 5.4 Study completion and participant withdrawal

*Participants may withdraw voluntarily from the study, or the Principal Investigator may discontinue a participant from the study. This section should state when a subject's participation in the study is complete and what circumstances would result in the withdrawal of the participant. However, because this is a cross-sectional, noninterventional study that does not involve follow-up, no protocol-prescribed criteria for withdrawal are described in the sample text below.*

For the diagnostic accuracy study, a participant is considered to have completed the study once they have completed the last step of the study procedures in which they are involved (i.e., once capillary and venous specimens have been collected and the participant has received their standard-of-care test results and been referred as per national guidelines).

A participant may withdraw voluntarily from study participation at any time without any impact on their care or treatment. Given the brief duration of participation in the study, and the fact that the study does not include any treatment intervention or follow-up, there are no protocol-prescribed criteria for participant withdrawal.

# 6. Study procedures

*In this section, all study-related procedures that participants and specimens will undergo during the clinical performance study should be described. Note that the protocol may provide a high-level discussion of procedures. Additional detail can be further provided in a Manual of Procedures (MOP) or in Standard Operating Procedures (SOPs).*

# 6.1 Diagnostic accuracy study

### 6.1.1 Recruitment, consent, and enrollment

Recruitment of patients who are presenting with a fever or a history of a recent fever will occur at [site]. At the facility, study staff trained in the protection of human subjects will approach individuals for prospective enrollment. Study staff will screen individuals for eligibility and ensure that they meet all study inclusion criteria and do not meet exclusion criteria. Recruitment will be conducted in such a way as to not interfere with the potential participant's care. The study team will explain the study and invite the person to participate, clarifying that they will have time to discuss their decision with family or health professionals who are not involved in the study. If the person expresses interest in participating in the study, they will be directed to a private space for consenting. Written consent will be obtained from all participants. For minors under the age of 18 years, parents [or legal guardians, if appliable] will consent to their child's participation; children aged [specify age of assent in study location] will also provide assent. See the detailed consent procedures and explanation of ethical considerations in sections 7 and 12.2. Once an individual provides consent to participate, they will be enrolled into the study. No study-specific procedures will be performed until written informed consent is collected and a participant identification number is assigned.

### 6.1.2 Procedures at the point of care

*To accompany this protocol, template Case Report Forms (CRFs) are also available from PATH.*

*For RDTs, it is recommended to ensure that test operators record results to the highest detail practicable—especially for the investigational product(s). For example, ideally, observations of test anomalies (e.g., red background, incomplete clearance, etc.) or test line problems should be recorded following standard definitions and example images should be provided during training. Resources to support this are available from PATH. Additionally, it is also recommended to record test and control line intensities using a standardized color chart. Consult with the test manufacturer when determining which color chart to use with a given product. Color scales should be standardized (i.e., printed with the same printer/ink) and stored securely, away from direct sunlight. As noted in WHO's Technical Guidance Series for WHO Prequalification – Diagnostic Assessment: Principles of Performance Studies (TGS-3), "Results recorded in this way are important for study validity in that they allow changes in IVD [in vitro diagnostic] performance to be better understood (e.g., signal degradation over time) than is the case for qualitative statements such as 'positive', 'negative' or 'all specimens passed'."*[iv]

*For venous blood collection, investigators should select an appropriate anticoagulant based on compatibility with the tests to be conducted using this specimen.*

Following the completion of informed consent and enrollment, study staff will collect basic demographic data (e.g., sex, age, ethnicity, pregnancy status) and relevant information on health status/medical history from participants. This information will be recorded on a Case Report Form (CRF). Appropriately trained study staff will obtain a capillary finger stick of approximately [specify volume; note: this will be dependent on the number of tests conducted using capillary specimens] µL from the participant. This sample will be used to perform the standard-of-care test for malaria [specify which] as well as [one or two] investigational

[iv] World Health Organization (WHO). *Technical Guidance Series for WHO Prequalification – Diagnostic Assessment: Principles of Performance Studies*. WHO; 2017. Licence: CC BY-NC-SA 3.0 IGO. https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf?sequence=1

RDT[s]. [Specify the volume of capillary blood required for all tests to be conducted at the point of care.] The RDT(s) require [number; typically 5] µL of blood specimen. [If microscopy is being conducted as the standard-of-care test, specify the volume required; typically around 20 µL of whole blood]. The finger-stick blood collection and testing with the investigational test[s] at the point of care will be performed in accordance with the products' Instructions for Use. Results of the RDTs performed on-site will be recorded on CRFs. All RDTs will be read according to product instructions, within the required time frames.

If an invalid RDT result is obtained at the point of care, the RDT will be repeated if the participant agrees to provide a new finger-stick sample. Each test will be repeated only once. Invalid results from any testing will be recorded in source documents by study staff.

A thermometer and a hygrometer will be placed at the study site near the assays. Temperature and humidity will be observed at the time the tests are run and recorded in the study database. A color chart and an RDT interpretation guide will be provided to standardize the rating of the test and control line intensity and the interpretation of results. Observations of test anomalies, such as background or test line problems, will also be recorded.

Results of the standard-of-care test will be delivered to patients by the health care workers as per standard routine. If a participant tests positive for malaria by the standard-of-care test, they will be referred for follow-up and case management in accordance with public health guidance.

Study staff who are trained in phlebotomy will also draw a [number] mL tube of venous blood from the participant with an appropriately sized vacutainer. All blood collection will be done in accordance with WHO guidelines.[13] The venous blood sample will be taken using a standard venipuncture kit, and blood will be collected in a blood collection tube with [specify which] anticoagulant and labeled with the participant's study identification number. Immediately after collection, whole blood in [anticoagulant] will be stored in a refrigerator or a cooler box and transported to the laboratory for further testing.

### 6.1.3 Laboratory procedures

*Handling of venous blood should follow best practices in instructions for all tests run. Limits on temperature and storage should be clearly identified and chosen for workflow feasibility and to preserve the specimen integrity. Volumes and numbers of aliquots should be chosen to minimize freeze-thaw or reuse of aliquots for reference, confirmatory, or repeat testing; they also should be appropriately sized for planned tests. All laboratory procedures and storage of samples should be clearly described in laboratory SOPs.*

*All test operators should be blinded to any prior test results and the clinical status of participants. As with capillary testing, it is recommended to ensure that RDT results are recorded to the highest detail practicable. Any color charts or guides used should be the same version and type as those used for capillary testing to allow for data comparability. Observations of test anomalies or test line problems should also be recorded systematically, according to standardized descriptions and example images provided during training.*

At the laboratory, aliquots of the venous blood will be prepared in cryogenic tubes and tested or frozen, as described in Table 1 below, within [number for handling] hours of collection.

**Table 1. Summary of study aliquoting procedures.**

| Aliquot | Volume | Description and purpose |
|---|---|---|

| | | |
|---|---|---|
| A | [Specify] | This aliquot will be stored fresh in the refrigerator at 2°C–8°C and kept refrigerated prior to testing for up to [specify time frame; within 24 hours is recommended] from collection time. This aliquot will be used for the following:<br>• Prepare study-specific research microscopy slides as per WHO procedures (approximately 20 µL).[14,15]<br>• Repeat the investigational test[s] ([5] µL per test).<br>• Conduct the comparator RDTs [list these] ([5] µL per test).<br>Any remaining specimens should be discarded once testing is complete, and no longer than [specify time frame; 2 weeks maximum] after collection. |
| B | [Specify] | Store frozen at −70°C or lower within 24 hours of collection. This frozen venous whole blood aliquot will be used for the reference PCR assay. Additional testing (e.g., to characterize *hrp2/hrp3* deletion status in *P. falciparum* infections) [may or will] be run on this aliquot or an additional frozen blood aliquot. |
| C | [Specify] | Store frozen at −70°C or lower within 24 hours of collection. This aliquot will be used for confirmatory or repeat testing. |
| D | [Specify] | Store frozen at −70°C or lower within 24 hours of collection. [Depending on volume and storage space, describe additional aliquoting procedures, as necessary.] |

Note: *hrp2*, histidine-rich protein 2 (gene); *hrp3*; histidine-rich protein 3 (gene); PCR, polymerase chain reaction; WHO, World Health Organization.

RDT testing using venous blood will be done according to instructions for use for each test. Each RDT used in the study will be performed by an independent operator blinded to any prior test results and the clinical status of participants. Operators performing microscopy and reference tests will be blinded to RDT results, and vice versa, so as to avoid diagnostic review bias. If an invalid RDT result is obtained, the RDT will be repeated. Each test will be repeated only once. Invalid results from any testing will be recorded in source documents by study staff.

A thermometer and a hygrometer will be placed at the study site near the assays. Temperature and humidity will be observed at the time the tests are run and recorded in the study database. For RDTs, a color chart and an RDT interpretation guide will be provided to standardize the rating of the test and control line intensity and the interpretation of results.

Study-specific research microscopy slides will be read by two qualified and certified microscopists. In the event of a discrepancy in their readings, as determined by the Obare Method Calculator, a third qualified and certified microscopist will conduct a third reading.[16]

### 6.1.4 Confirmatory and additional testing

*Describe plans for confirmatory testing, including the location of such testing, if different from the primary study site.*

Designated aliquots will be stored for additional or repeat testing to resolve discordant results as well as additional possible confirmatory testing. Confirmatory testing of stored blood may include typing and sequencing of *Plasmodium* genes encoding antigens of interest, including but not limited to HRP2, HRP3, and pLDH. Investigational or comparator RDTs may also be repeated on frozen specimens. Confirmatory testing of stored blood may also include a quantitative antigen immunoassay to measure concentrations of *Plasmodium* antigens, including but not limited to HRP2, HRP3, and pLDH. Confirmatory testing will be conducted at [location].

### 6.1.5 Specimen storage

*If the study involves establishment of a biorepository through the storage of specimens for use in future research, this should be clearly described in the protocol as well as in agreements between the sponsor and clinical site. Additionally, a biorepository governance plan should be established to ensure appropriate long-term oversight of specimens for future use.*

At study close, leftover specimens will be stored in a biorepository at [location] for possible testing related to [specify scope of future testing] as part of future studies. If the stored specimens will be used for purposes other than those described in this protocol, [institution] will be responsible for obtaining the necessary ethical approvals. Specimens stored for future use will be coded to avoid identification of the subject. Stored specimens will be destroyed after a maximum of [number] years after the end of the study.

Specimens sent to [location] for confirmatory testing will be discarded at the close of the study.

## 6.2 Usability study

### 6.2.1 Recruitment, consent, and enrollment

Participants in the usability study will be purposively recruited from lay providers and health care workers in [location] who provide malaria case management services and would be considered intended end users of malaria RDTs. Eligible individuals will be invited to participate. A study staff member trained in the protection of human subjects will explain the objectives of the usability assessment and what participation will entail. The study staff will also clarify that the potential participant will have time to discuss their decision with family or people who are not involved in the study. Study staff will explain that participation is voluntary and that it will not affect the nature of their employment. The voluntary nature of participation in the usability component will be emphasized. Interested and eligible individuals will be consented to participate in the study. Consent will take place in a private setting in the health facility. See the detailed consent procedures and explanation of ethical considerations in sections 7 and 12.2.

### 6.2.2 Training and data collection

*If more than one investigational test is under evaluation, it is recommended that each participant evaluate the usability of only one product (i.e., a separate usability study should be conducted for each test). This will ensure that there is no confusion among participants regarding features of the test under evaluation.*

*For the contrived tests, studies could use either (1) photos of test results or (2) physical cassettes with modified paper inserts to simulate results. It is not recommended to use the same graphical images of test results that appear on the Instructions for Use for the product(s) under evaluation. Advantages, disadvantages, and implementation considerations for each approach are described below.*

| ***Contrived test option*** | ***Advantages*** | ***Disadvantages*** | ***Considerations for implementation*** |
|---|---|---|---|
| ***Photos*** | *Reflective of real-world test results.* | *Some combinations of results (especially invalid results) are rare. Obtaining images with the right combinations of results may be challenging.* | *If photos are used across multiple sites, centralized printing is recommended to ensure data comparability across studies.* |

| | | | |
|---|---|---|---|
| | | *Weak lines can be difficult to see in photos.*<br>*Print quality can be variable.* | *Some image manipulation may be required to obtain rare results.* |
| ***Physical cassettes with modified inserts*** | *Can be standardized across sites/studies to ensure data comparability.*<br>*Not subject to differences in print/screen quality.* | *Requires shipment to sites.*<br>*Less reflective of real-world test results.* | *Ensure sufficient quantity is available for the study sample.* |

Following consent, participants will receive training on the use of the investigational test. Training content will be developed and delivered in collaboration with the relevant health administrators at the study site and the test manufacturer. Following the training, participants will then be given a questionnaire to assess test label comprehension and results interpretation. At that time, the study staff will emphasize that the questionnaire is being used to assess the effectiveness of the training content and test instructions—not the skills or performance of the user. The questionnaire will be designed to assess the ability of intended users to correctly comprehend key messages from packaging and labeling: key warnings, limitations and/or restrictions, proper test procedure, and test result interpretation. Usability participants will also interpret the results of contrived in vitro diagnostics (IVDs; e.g., static/premade tests) to assess their ability to correctly interpret predetermined test results. Contrived tests will be made to demonstrate the following potential test results: nonreactive, range of invalid results, reactive, weak reactive. Tests with more than one test line should have all possible combinations represented in results.

## 6.3 Summary of study procedures

Study procedures and estimated time requirements are summarized in Table 2 below, by participant group.

**Table 2. Summary of study procedures and time requirements, by participant group.**

| Participant group | List of study procedures (estimated time) |
|---|---|
| Children aged 2–17 years (diagnostic accuracy study) | • Assent (if applicable), parental consent, and enrollment (20 minutes).<br>• Demographic questionnaire (10 minutes).<br>• Point-of-care tests and blood draw (up to 30 minutes). |
| Adults aged 18+ years (diagnostic accuracy study) | • Consent and enrollment (20 minutes).<br>• Demographic questionnaire (10 minutes).<br>• Point-of-care tests and blood draw (up to 30 minutes). |
| Lay providers/health care workers (usability study) | • Consent and enrollment (20 minutes).<br>• Training on the use of the investigational test[s] (up to 1 hour).<br>• Usability assessment questionnaire (45 minutes). |

# 7. Consent

*The protocol should include a description of the general process for obtaining informed consent. This should include a description of the informed consent process for minors and illiterate individuals.*

## 7.1 Diagnostic accuracy study

Written informed consent or assent will be obtained for all participants prior to any study procedures. Consent will occur in a private place at the health care facility. Members of the study team trained in the protection of human subjects will conduct the consent procedure. Study staff will review the study details with the potential participant and invite the person to participate, clarifying that they will have time to discuss their decision with family or health professionals who are not involved in the study. The potential participant will be given an opportunity to review the informed consent form and ask questions. Consent will take place in [language], and the informed consent form will be prepared in English and translated into [language]. Parent [or legal guardian, if applicable] permission will be obtained for all participants under the age of 18 years. As per local requirements, child assent will be obtained for all participants between the ages of [specify]. Children aged [specify] will express their assent in writing in the presence of their parent as a witness to ensure the assent process is without any coercion. When appropriate, a conversational style oral presentation of consent information will be made in the local language, matching the information written, to participants in order to account for any difficulties understanding written consent forms due to low literacy.

During the consent process, the study team will explain the purpose of the study and what involvement will entail. It will be emphasized that participation is voluntary and that their decision will not negatively affect the care they receive in any way. The informed consent form will review the study purpose, the procedure involved in participation, the potential participant's rights to withdraw, confidentiality, and benefits and risks of participating in the study. The potential participant will have the opportunity to ask questions. Consent for participation will be documented on a written informed consent form. One copy of the signed informed consent form will be provided to the participant, and one copy will be kept for study records.

If a potential participant is illiterate, an independent, impartial literate witness will be asked to join the consent process. This witness will be a health care worker or other family member uninvolved in the study. The study staff will read the consent form aloud to the potential participant, and the witness will verify that the information read aloud matches the information written on the consent form. The witness will affirm that the study participant chose to be in the research study, that they were present the whole time the study was being explained, and that the participant had a chance to ask questions. The participant will get a copy of this form to keep. The witness will also sign the consent form.

## 7.2 Usability study

Consent will take place in a private setting in the health facility after lay providers/health care workers have been recruited. Members of the study team trained in the protection of human subjects will conduct the consent procedure. During the consent process, the study team will explain the purpose of the study and what involvement will entail. It will be emphasized that participation is voluntary and that the potential participant's decision will not affect their employment in any way. The potential participant will have the opportunity to ask questions. Consent for participation will be documented on a written informed consent form. One copy of the signed informed consent form will be provided to the participant, and one copy will be kept securely for study records.

# 8. Study products

Table 3 summarizes the study products to be included in the diagnostic accuracy study.

**Table 3. Summary of study products.**

| Test type | Test full name | Manufacturer | Catalog number | Registration status at study site | Sample type | Location of use | Expected blood volume required |
|---|---|---|---|---|---|---|---|
| Investigational | [Name] | [Manufacturer] | [Cat no] | [Specify] | Capillary | Point of care | 5 µL |
| | | | | | Venous | Laboratory | 5 µL |
| Investigational | [Name] | [Manufacturer] | [Cat no] | [Specify] | Capillary | Point of care | 5 µL |
| | | | | | Venous | Laboratory | 5 µL |
| Comparator—standard-of-care test | [Name] | [Manufacturer] | [Cat no] | Registered | Capillary | Point of care | [Specify] |
| Comparator | [Name] | [Manufacturer] | [Cat no] | Specify] | Venous | Laboratory | 5 µL |
| Comparator | [Name] | [Manufacturer] | [Cat no] | [Specify] | Venous | Laboratory | 5 µL |
| Comparator | Microscopy (study-specific research grade) | | | Registered | Venous | Laboratory | 20 µL |
| Reference | PCR [Name] | [Method] | [Targets and reference] | [Specify] | Frozen venous | Laboratory | [Specify] |
| Confirmatory | *hrp2/hrp3* gene deletion assay [Name] | [Method] | [Targets and reference] | [Specify] | Frozen venous | Laboratory | [Specify] |
| Confirmatory | Quantitative antigen assay | | | Not registered | Frozen venous | Laboratory | [Specify] |

Note: cat no, catalog number; *hrp2*, histidine-rich protein 2 (gene); *hrp3*, histidine-rich protein 3 (gene); PCR, polymerase chain reaction.

## 8.1 Investigational test[s]

### 8.1.1 Description of investigational test[s]

*Describe the investigational test(s) to be used in the study. Product information should be obtained from the Instructions for Use and other device labeling. For malaria RDTs, it is recommended to include the following information at minimum: full product name, name and address of the manufacturer, catalog number, kit components and description of each component, target malaria species and antigen(s), intended use, principle of the assay, indicated specimen types, packaging and labeling, description of how results are interpreted, and commercial/regulatory status in the country where the evaluation is taking place. Additionally, any relevant preclinical evidence, including performance claims, can also be summarized.*

#### 8.1.1.1 [Name of investigational test 1]

The [test name] ([manufacturer name], [country], [catalog number]) is a sensitive rapid chromatographic immunoassay for the qualitative detection of [specify; e.g., HRP2 and pLDH of *P. falciparum* on one test line and of pLDH of *P. vivax* malaria on a second test line] in human whole blood (capillary or venous in [anticoagulant]). This test is a lateral flow test for in vitro professional diagnostic use and is intended as an aid to early diagnosis of malaria infection in patients with clinical symptoms. The manufacturer reports a sensitivity of [specify] and a specificity of [specify] for *P. falciparum* and a sensitivity of [specify, if applicable] and a specificity of [specify, if applicable] for *P. vivax.* This test is not commercially available and is not registered for use in [country]. Results indicating positive antigen detection will not be used to inform treatment.

#### 8.1.1.2 [Name of investigational test 2, if applicable]

[See sample text above]

### 8.1.2 Acquisition and accountability

*State how the investigational product(s) will be provided to the site. Describe plans for the storage and use of the investigational product(s), as well as plans for disposal of expired products or return of unused products. Additional detail can be provided in a study-specific SOP or MOP, and an investigational product accountability log may be used.*

The investigational product[s] will be provided by the sponsor to the sites, which will coordinate shipments. The local study lead will be responsible for obtaining appropriate import permits. Transportation of the investigational product[s] will adhere to the conditions required in the Instructions for Use, and monitoring of these conditions during transport will be undertaken. The local study lead is responsible for investigational product accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). Investigational product accountability logs filled at each site will ensure the proper follow-up of the used, failed, and remaining tests.

### 8.1.3 Storage and handling

*Describe storage and stability requirements of the investigational product(s) (e.g., light exposure, temperature, humidity conditions). For malaria RDTs, include any relevant use instructions.*

The investigational product[s] will be stored in a secure, environmentally controlled, and monitored area in accordance with the labeled storage conditions, with access limited to the investigator and authorized site staff. The investigational product[s] will be stored during the study in accordance with the instructions for use, which specifies [insert storage requirements]. If the test kit or any components are stored refrigerated (2°C–8°C), they should be brought to room temperature (18°C–30°C) prior to testing. Testing will be performed in accordance with the manufacturer's instructions.

### 8.1.4 Quality controls

*Describe quality control procedures to be used with the investigational product(s) during the study.*

*WHO International Standards can be used for RDT quality control checks at defined study time points. Protocols for this purpose are available from PATH.*

Upon arrival, a quality check should be conducted for each lot of tests used in the study using the WHO International Standards for *P. falciparum* (NIBSC code: 16/376) and *P. vivax* (NIBSC code: 19/116) antigen which have been prepared according to each standard's instructions and standard operating procedures (SOPs) to support quality testing. New lots may be used only after this quality check has been successfully completed. The quality check will be repeated at predetermined time point(s) during study implementation, in accordance with the SOPs/manual of procedures (MOP) (e.g., midpoint and at study end).

## 8.2 Comparator tests

*Describe the comparator test(s) to be included in the study. For each comparator RDT, similar information as above should be provided. The rationale for the selection of the comparator test(s) should be justified in the protocol.*

### 8.2.1 Research-specific light microscopy

*Research-specific light microscopy for malaria should follow procedures outlined in WHO's methods manual.[ii] Dhorda et al. (2020)[v] is also a relevant resource to support this.*

*Researchers may also wish to use the Obare Method Calculator, a tool to support calculating mean parasite density and assessing whether or not two blood samples are concordant. It is a Microsoft Excel–based tool developed to facilitate adherence to the recommendations for internal quality control in WHO's methods manual for research microscopy for malaria. It is available from https://www.wwarn.org/tools-resources/procedures/obare-method-calculator.*

Light microscopy is a standard of practice for malaria diagnosis. Giemsa-stained thick blood films are used for detection of *Plasmodium* parasites, whereas thin blood films enable identification of infecting species. However, misclassification of *Plasmodium* species by malaria microscopy is common, and studies have demonstrated low sensitivity, which make it a sub-optimal reference test.[17,18] Therefore, light microscopy is used as a comparator test in this study, rather than a reference test.

Study-specific research-grade light microscopy will be performed for *Plasmodium* infection detection, quantification, and discrimination.[19] This will be performed on thick and thin blood slides at the clinical site by two qualified and certified readers, as well as a third qualified and certified reader using Obare in the case of a discrepancy in the initial readings. Study sites will ensure that, at a minimum, a study-grade (equivalent to WHO best practice) light microscopy slide is run, using appropriate quality control procedures.[14,15,16]

### 8.2.2 [Name of comparator RDT 1, standard of care]

*Note: This example is written assuming the standard-of-care RDT.*

The [test name] ([manufacturer name], [country], [catalog number]) is a sensitive rapid chromatographic immunoassay for the qualitative detection of [specify; e.g., HRP2 of *P. falciparum* on one test line and of pLDH of *P. vivax* malaria on a second test line] in human whole blood (capillary or venous in [anticoagulant]). This test is a lateral flow test for in vitro professional diagnostic use and is intended as an aid to early diagnosis of malaria infection in patients with clinical symptoms. The manufacturer reports a

[v] Dhorda M, Ba EH, Baird JK, et al. Towards harmonization of microscopy methods for malaria clinical research studies. *Malaria Journal*. 2020;19(1):324. doi:10.1186/s12936-020-03352-z

sensitivity and a specificity of [specify] and [specify] for *P. falciparum* and [specify] and [specify] for *P. vivax.*

This test is commercially available and is registered for use in [country]. This test is the current standard-of-care test for malaria at [facility]. It will be run on [specify specimen type] samples for participants in the study. As per the standard of care, the test will be used to inform clinical management of study participants and referrals to the health system for treatment in the event of a positive result.

### 8.2.3 [Name of comparator RDT 2, if applicable]

The [test name] ([manufacturer name], [country], [catalog number]) is a sensitive rapid chromatographic immunoassay for the qualitative detection of [specify; e.g., HRP2 of *P. falciparum* on one test line] in human whole blood (capillary or venous in [anticoagulant]). This test is a lateral flow test for in vitro professional diagnostic use and is intended as an aid to early diagnosis of malaria infection in patients with clinical symptoms. The manufacturer reports a sensitivity and a specificity of [specify] and [specify] for *P. falciparum* [as needed: and [specify] and [specify] for *P. vivax*].

This test is commercially available and is registered for use in [country]. This test will only be conducted in the laboratory on venous specimens and will not be used to inform treatment decisions. This test is WHO prequalified (reference number) and is included in the study as a conventional comparator RDT.

### 8.2.4 [Name of comparator RDT 3, if applicable]

[See sample text above]

## 8.3 Reference test

*PCR is recommended as the reference assay as this method has been shown to be highly sensitive and specific for the diagnosis of malaria, allowing for a limit of detection (LOD) ranging from 0.7 to 10 parasites per µL.*[vi] *PCR is the preferred reference method as it minimizes classification bias due to misdiagnosis of infecting species, and it has shown superior performance over microscopy. The primary endpoints for this protocol assume PCR as the reference assay. The protocol should be modified if alternate reference testing is proposed. The selection of the reference method for the study should be clearly articulated and justified in the protocol.*

*Numerous PCR protocols and methods exist. Real-time, quantitative polymerase chain reaction (qPCR) for quantification and speciation is preferred over qualitative PCR given its potential for greater sensitivity and specificity, reproducibility, lowered risk for contamination, and quantitative output, which can be correlated to parasite density. The qPCR method should demonstrate high analytical sensitivity, with an LOD from 1 to 10 parasites per µL for P. falciparum using both genus- and species-specific primers, and comparable sensitivity for P. ovale, P. malariae, and P. vivax. Duplexing the assay for simultaneous detection of Plasmodium genus and specific species in a single reaction will make it well suited for high-throughput applications. A probe-free format using fluorescence-tagged primers further simplifies assay setup and reduces cost. WHO International Standards can be used to harmonize qPCR results across different methods and testing sites, and to establish the assay's LOD. Standardized protocols to support this process are available from PATH.*

[vi] Lucchi NW, Narayanan J, Karell MA, et al. Molecular diagnosis of malaria by photo-induced electron transfer fluorogenic primers: PET-PCR. *PLOS One*. 2013;8(2):e56677. doi:10.1371/journal.pone.0056677

A quantitative, real-time PCR assay for *Plasmodium* detection and speciation will be used as the reference assay for this study. This assay will be performed by qualified and trained laboratory personnel at [laboratory location] and under appropriate quality procedures to ensure data comparability across studies. Specifically, the [specify name] assay will be run, as described by [include relevant citations]. Briefly, [describe methods in brief].

## 8.4 Confirmatory tests

### 8.4.1 Assay for *hrp2/hrp3* deletion characterization in *P. falciparum* infections

*Depending on the goals/objectives of the study and the epidemiology at the study location, conducting confirmatory testing for the hrp2/hrp3 gene deletion status of P. falciparum infections may be advisable. Specimens with discordant profiles (e.g., confirmed positive for P. falciparum on PCR but negative on an HRP2-based P. falciparum RDT) may be suggestive of potential deletions. A high number of false-negative HRP2-based RDT results or a known prevalence of hrp2/hrp3 deletions at the study site may signal the value of confirmatory testing for deletions. In general, it is preferred for all PCR-positive specimens with confirmed P. falciparum to be tested for deletion status.*

*Numerous methods exist for molecular confirmation of deletion status; these are described in Beshir et al. (2022).*[vii] *These include conventional PCR, multiplex real-time PCR, digital PCR, and next-generation sequencing, each with advantages and disadvantages. If data from the study are intended to be submitted to WHO for prequalification, confirmation of hrp2/hrp3 deletion status by sequencing is recommended. If PCR is used, it is recommended to employ a validated protocol that has been verified in the performing laboratory, with an established LOD, clear cycle threshold cutoff values, employment of human housekeeping genes to evaluate specimen integrity, and appropriate quality controls.*

The *hrp2/hrp3* deletion status of *P. falciparum* infections will be confirmed by [method]. This assay will be performed by qualified and trained personnel at [laboratory] and under appropriate quality control procedures to ensure data comparability across studies. [Describe specific methodology, including appropriate citations].

### 8.4.2 Malaria antigen quantification assay

*A quantitative antigen assay will allow for comparison of the antigen-based RDT results against that of a quantitative assay that detects the cognate analyte. While not required for WHO prequalification as a reference in clinical studies, this testing could be incorporated into a study for several purposes:*

- ***As a secondary reference test:*** *This would allow for comparison of RDT results against the cognate antigen as determined through the quantitative assay and calculation of corresponding diagnostic performance metrics (e.g., sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV]). For this purpose, all study specimens (including PCR negatives) should be tested using the quantitative antigen assay.*
- ***As a confirmatory test to understand discordant results from the primary evaluation:*** *For this purpose, it is recommended that all specimens testing positive for malaria on any assay*

[vii] Beshir KB, Parr JB, Cunningham J, Cheng Q, Rogier E. Screening strategies and laboratory assays to support *Plasmodium falciparum* histidine-rich protein deletion surveillance: where we are and what is needed. *Malaria Journal*. 2022;21:201. doi:10.1186/s12936-022-04226-2

*(investigational, comparator, and/or reference), as well as a subset of negatives, undergo confirmatory antigen quantification.*

*Several methods are available for quantitative antigen testing. Commercial assays include the Q-Plex Human Malaria Array (Quansys Biosciences) and Quantimal CELISA (Cellabs). Assays using the Luminex platform have been reported in literature or are under development. As validated protocols are made public, they will be referenced here.*

A research-use-only quantitative assay to measure malaria [specify; e.g., HRP2] and [specify; e.g., pLDH] antigens may be conducted as a confirmatory test.

# 9. Statistical considerations

## 9.1 Sample size

*The diagnostic accuracy study's sample size calculation should be informed by the study's primary objectives/endpoints, design, and desired power and should be clearly justified in the protocol. Several possible and common approaches for sample size estimation are described below for a paired, cross-sectional design where all participants undergo testing with the same investigational, comparator, and reference tests. However,* ***it is recommended to consult a statistician to ensure that clinical studies are appropriately designed and powered.***

***Option 1: Setting a prevalence-based sample size to obtain a predefined target number of positive specimens for a given species.***

*Some bodies, for example WHO Prequalification, require that the clinical performance of malaria RDTs be established using specific numbers of confirmed positive specimens. According to WHO's Technical Specification Series (TSS) guidance document for submission of malaria RDTs for WHO prequalification, the TSS-3,*[viii] *the following is required:*

- *Products intended for the detection of P. falciparum should be evaluated with at least* ***400 confirmed P. falciparum–positive*** *specimens from a symptomatic population for sensitivity.*
- *Products intended for the detection of P. vivax should be evaluated with at least* ***100 confirmed P. vivax–positive*** *specimens for sensitivity.*
- *For products making a claim for "pan-specific" detection of Plasmodium species, performance should be determined for each species for which specimens are available, which should include P. falciparum and P. vivax at a minimum.*
- *Products should be evaluated with at least* ***1,000 Plasmodium-negative*** *specimens from a symptomatic population for specificity.*
- *RDTs detecting the LDH antigen should be evaluated with prospective sampling of gene deletion specimens according to most current version of the TSS-3. As of May 2025, the current draft TSS-3*

[viii] World Health Organization (WHO). *Technical Specifications Series for Submission to WHO Prequalification – Diagnostic Assessment. TSS-3: Malaria Rapid Diagnostic Tests*. Licence: CC BY-NC-SA 3.0 IGO. WHO; 2017. https://apps.who.int/iris/bitstream/handle/10665/255038/9789241512275-eng.pdf

*revision*[ix] *specifies* ***30 specimens with hrp2/hrp3 gene deletions****, with* ***at least 20 being double deletions*** of both hrp2 and hrp3.

*In view of the manufacturer's clinical strategy and the portfolio of studies being conducted for a given product and/or number of sites under a multicountry protocol, a target number of P. falciparum and/or P. vivax samples may be set for an individual study or site to contribute toward an overall data package that aims to fulfill these or other relevant requirements. With this approach, a study's overall target sample size would be set based on the expected prevalence of malaria, by species, at the study site. This sample size would supersede any requirements related to statistical power. Depending upon available resources and observed prevalence during the recruitment period, the study could either recruit until the target is met for a specific malaria species or recruit until a total overall sample size is met. The latter is the approach taken in the example text provided below.*

***Option 2: Setting a statistically informed sample size that would allow for the estimation of an individual test's clinical sensitivity or specificity with a desired level of precision.***

*One of the most commonly used methods for determining sample size in diagnostic accuracy studies is based on estimating sensitivity and/or specificity with a predefined level of precision. This approach is typically used when the aim is to obtain a sufficiently narrow confidence interval around the estimate of sensitivity and/or specificity. The required number of diseased subjects (for sensitivity) or nondiseased subjects (for specificity) is calculated using a standard formula that adjusts for the expected disease prevalence in the population and considers the anticipated performance of the investigational product(s). Relevant references for this approach include Buderer (1996),*[x] *Hajian-Tilaki (2014),*[xi] *and Akoglu (2022).*[xii]

***Option 3: Setting a statistically informed sample size that would allow for the comparison of sensitivities and/or specificities of two tests (e.g., investigational and comparator RDTs).***

*When a study aims to compare the diagnostic performance (e.g., sensitivity and/or specificity) of two tests in the same population under a paired design, the sample size for the study can be set to ensure sufficient statistical power for this comparison (e.g., through a McNemar's test for paired proportions). Relevant references for this approach include Akoglu (2022),*[xii] *Connor (1987),*[xiii] *and Tang et al. (2002).*[xiv] *It is recommended to consult a statistician and ensure that underlying assumptions regarding investigational and comparator test performance are robust and thoroughly considered if using this method.*

*For the qualification of usability, it is recommended to include approximately 15 lay providers/health care workers per site for each investigational product under evaluation. The TSS-3 for submission of malaria RDTs for WHO prequalification specifies that at least ten intended users from two geographically diverse populations should be included.*

---

[ix] World Health Organization (WHO). *Draft for Comment: Technical Specifications Series for Submission to WHO Prequalification – Diagnostic Assessment. TSS-3: Malaria Rapid Diagnostic Tests*. 2nd ed. Geneva, Switzerland: World Health Organization; 2023. https://extranet.who.int/prequal/sites/default/files/document_files/draft_for_comment_2nd_edition_TSS_3_Malaria_RDTs.pdf

[x] Buderer NMF. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. *Academic Emergency Medicine*. 1996;3(9):895–900. doi:10.1111/j.1553-2712.1996.tb03538.x

[xi] Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. *Journal of Biomedical Informatics*. 2014;48:193–204. doi:10.1016/j.jbi.2014.02.013

[xii] Akoglu H. User's guide to sample size estimation in diagnostic accuracy studies. *Turkish Journal of Emergency Medicine*. 2022;22(4):177–185. doi:10.4103/2452-2473.357348

[xiii] Connor RJ. Sample size for testing differences in proportions for the paired-sample design. *Biometrics*. 1987;43(1):207–211.

[xiv] Tang ML, Tang NS, Chan ISF, Chan BPS. Sample size determination for establishing equivalence/noninferiority via ratio of two proportions in matched-pair design. *Biometrics*. 2002;58(4):957–963. doi:10.1111/j.0006-341x.2002.00957.x

The sample size for this study is based on the expected prevalence of *P. falciparum* and *P. vivax* at the study site and the data requirements set by WHO Prequalification.[20] For RDTs intended for detection of *P. falciparum*, WHO specifies that clinical performance studies should include at least 400 confirmed *P. falciparum*–positive specimens from a symptomatic population. For RDTs intended for detection of *P. vivax*, WHO specifies that clinical performance studies should include at least 100 confirmed *P. vivax*–positive specimens. All products should be evaluated for specificity with at least 1,000 *Plasmodium*-negative specimens.

This study aims to enroll at least [number] participants that are PCR-positive for *P. vivax* malaria and [number] participants that are PCR-positive for *P. falciparum* malaria. Data from the study will be combined with results from other clinical evaluation sites in other geographies in order to fulfill WHO Prequalification requirements. The anticipated prevalence of *P. vivax* and *P. falciparum* malaria are [specify, citing relevant literature and/or clinical records to justify the estimate]. Accounting for a low anticipated dropout rate for participants with difficult venous blood collection, we expect that a sample size of [number] will be sufficient to achieve the study's target numbers of *P. falciparum* and *P. vivax* malaria cases at prevalence.

Per guidance from WHO Prequalification, [number] lay provider/health care worker participants who are intended end users of malaria RDTs will be purposively sampled for the usability assessment [for each investigational test]. As in the diagnostic performance assessment, the data from this usability assessment will be combined with data from a similar sample of lay providers/health care workers in other evaluations in order to reach the target number of users required by WHO Prequalification.

The expected overall sample size for this study, by population, is summarized in Table 4.

**Table 4. Summary of study sample size.**

| Participant group | Sample size |
|---|---|
| Febrile patients (diagnostic accuracy study) | [number] |
| Lay providers/health care workers (usability study) | [number] |

## 9.2 Statistical analysis

*The protocol should include a description of planned statistical methods for evaluating the stated endpoints. This section may be supplemented by a more detailed statistical analysis plan, if required, which should be developed prior to the initiation of recruitment. As per ISO 20916:2019, either the protocol or statistical analysis plan should, at a minimum, contain the following information:*

- *Statistical design, method, and analytical procedures.*
- *Sample size justification (see section 9.1).*
- *Level of significance and power of the clinical study (see section 9.1).*
- *Pass/fail acceptance criteria to be applied to the results of the clinical study.*
- *Provision for an interim analysis, if applicable.*
- *Procedures that ensure that all the data are taken into account.*
- *Treatment of missing, unused, or spurious data.*

*In the sections below, we provide general text relevant to statistical analysis for diagnostic accuracy and usability. Note that this protocol template assumes that PCR is the reference method used for all*

*performance analyses. Investigators may wish to include either primary or secondary analyses using other tests as the reference method (e.g., research microscopy or a composite reference that accounts for both PCR and research microscopy results). The selection of the reference method for the study should be clearly articulated and justified in the protocol.*

### 9.2.1 Diagnostic accuracy study

#### 9.2.1.1 Analytical population

All participants with valid reference assay results will be included in the final analytical population. A participant will be considered non-evaluable for the purposes of this study if:

- They withdraw consent for study participation before completion of the study procedures (i.e., before venous blood is collected).
- Inadequate venous blood sample(s) is obtained.
- The venous specimen was not stored or transported appropriately for processing and freezing (e.g., the sample was received outside the window described by the laboratory SOPs).
- No valid reference test result is obtained.

#### 9.2.1.2 Invalid or missing results

Missing capillary or venous investigational or comparator test results will be excluded from performance analyses.

Invalid results on all tests will be recorded. For RDTs, if an invalid result is obtained during the initial run, the test will be repeated if feasible. If an invalid result is obtained a second time, the final result will be recorded as invalid. Invalid results will be analyzed separately in the final performance calculations.

Invalid rates will be calculated for all RDTs. To calculate invalid rates, device failure percentages will be calculated as the total number of invalid tests divided by the total number of tests performed and then multiplied by 100%.

#### 9.2.1.3 Descriptive analyses

Data will be entered into a database with built-in validation rules to minimize data entry errors. Descriptive statistical analysis—including calculating point estimates, distribution, and frequencies of responses—will be used to summarize and characterize the study population. The number and percentage of participants infected with malaria, by species, according to all study assays will be determined. The distribution of parasite densities and parasite-specific antigen concentrations will also be described through density plots and histograms. All analyses will report 95% confidence intervals, when appropriate, using the [specify] method.

#### 9.2.1.4 Diagnostic performance endpoint analyses

The performance of the investigational and comparator tests on various sample types will be determined by calculating the sensitivity, specificity, PPV, and NPV as components of diagnostic reporting accuracy. Test results will be classified as either positive or negative.

Each test line result will be categorized as positive or negative and compared against the reference PCR assay as the gold standard for true positive and true negative. Test performance will also be presented for each specimen type (finger-stick versus venous blood), where applicable.

The test result classification, using PCR as the reference assay, is summarized in Tables 5 and 6.

**Table 5. *Plasmodium falciparum* test result classification.**

| ***Pf* test line results on RDT** | **PCR positive for *Pf*** | **PCR negative for *Pf*** |
|---|---|---|
| **Positive for *Pf* antigen(s) on RDT** | True positive (TP) | False positive (FP) |
| **Negative for *Pf* antigen(s) on RDT** | False negative (FN) | True negative (TN) |

Note: PCR, polymerase chain reaction; *Pf*, *Plasmodium falciparum*; RDT, rapid diagnostic test.

**Table 6. *Plasmodium vivax* test result classification.**

| ***Pv* test line results on RDT** | **PCR positive for *Pv*** | **PCR negative for *Pv*** |
|---|---|---|
| **Positive for *Pv* antigen on RDT** | True positive (TP) | False positive (FP) |
| **Negative for *Pv* antigen on RDT** | False negative (FN) | True negative (TN) |

Note: PCR, polymerase chain reaction; *Pv*, *Plasmodium vivax*; RDT, rapid diagnostic test.

Sensitivity will be determined by the following method:

TP = test and true positive (positive by PCR for *P. falciparum* or *P. vivax* and positive by the relevant RDT test line).

FN = false negative true positive (positive by reference PCR and negative by the relevant RDT test line).

Sensitivity = TP / (TP + FN).

Specificity will be determined by the following method:

FP = false positive (negative by reference PCR and positive by the relevant RDT test line).

TN = true negative (negative by reference PCR and negative by the relevant RDT test line).

Specificity = TN / (TN + FP).

Additionally, positive and negative predictive values will also be calculated according to the following methods:

Positive predictive value: TP / (TP + FP).

Negative predictive value: TN / (TN + FN).

This study is not intended to independently test the equivalence, non-inferiority, or superiority of the index tests compared to the comparator and/or standard diagnostic tests. Diagnostic performance indicators for each test will be presented separately.

#### 9.2.1.5 *hrp2/hrp3* deletion analyses

The proportion of *P. falciparum* infections with confirmed *hrp2/hrp3* deletions will be reported. Rates of false positives and false negatives observed on both the investigational and comparator tests will be reported. For the purposes of calculating sensitivity of the RDTs on specimens with *hrp2/hrp3* deletions, specimens with confirmed deletions will be considered as “true positives.”

#### 9.2.1.6 Acceptance criteria

[State any applicable acceptance criteria as appropriate.]

### 9.2.2 Usability study

The usability questionnaires will include both multiple choice and open-ended questions. Survey questions will be used as a framework for the analysis. Participants will be encouraged to comment on any aspects of the label or results they find confusing or inadequate. Analyses will include descriptive statistics and a tabular presentation of findings. [State any applicable acceptance criteria as appropriate.]

# 10. Data management

*The protocol should include a plan to be followed for managing data, including access to source data and the extent to which source data will be verified. As appropriate, the protocol may be supplemented by a study-specific data management plan. Example language is included below.*

## 10.1 Data entry and handling

The site should maintain adequate and accurate source documents and records that include all pertinent observations on each of the site's participants. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary. Source documents should be filed at the site.

All study data will be recorded on standard paper or electronic CRFs and test result worksheets. All data will be tracked by the participant's study identification number, which can be linked back to the participant's name via a linking log that will be securely maintained by [site].

All data will be entered into an electronic, password-protected study database with built-in validation rules to minimize data entry errors in accordance with SOPs. Data entry will be performed by staff at [site]. In settings where paper CRFs are used, site staff will be responsible for entering their data into the database. Detailed timelines for data entry will be described in the Data Management Plan and relevant SOPs. Data will be routinely verified for accuracy in relation to source documentation ("cleaned") throughout the study (see sections 10.2 and 11).

## 10.2 Data monitoring and management

Data management procedures for the study, including database setup, checks, and querying, will be described in a data management plan. The site is responsible for verifying that data entries are accurate and correct. Data will be evaluated for compliance with the protocol and for accuracy in relation to source documents (see section 11).

## 10.3 Confidentiality, data protection, and privacy

The investigators and staff involved with this study will comply with relevant laws relating to the confidentiality, privacy, and security of the participants' health information. The investigators will ensure that all participants' confidentiality is maintained. The participants will be identified by a unique study

identification number. The participants' names and any other identifying detail will not be included in the electronic study database. Participants will not be identified in any publicly released reports of this study. All records will be kept confidential by [site]. [Sponsor] will not have access to any records that identify the research participants. [Site] will maintain a secure linking log that links participants' study identification number to their name and contact information. All study data will be used and managed in accordance with local data protection requirements.

## 10.4 Data access

The log linking each participant's name and contact information to their study identification number will be maintained by the staff at [site]; the [sponsor] will not have access to the log. All records will be kept locked, and all databases will be password protected such that clinic staff and study staff will have access only to their respective databases.

Direct access for study-related monitoring, audits, and inspections will be granted to authorized representatives from the sponsor, host institutions, and the regulatory authorities.

Data-sharing and access terms are outlined in the Clinical Study Agreement between the sponsor and the site.

## 10.5 Data and specimen storage

[Site] will maintain, and store securely, complete, accurate, and current study records throughout the study. In accordance with regulations, study staff will retain all study records on-site for at least [number] years after study closure. Study records will not be destroyed prior to receiving approval for record destruction from the sponsor. Applicable records include source documents, site registration documents and reports, informed consent forms, and notations of all contacts with participants.

In line with GCP and sponsor guidelines, electronic data generated from the clinical study will be kept at [location] after the study has ended. Electronic study records will be de-identified upon completion of data collection. The electronic records will be maintained indefinitely in the databases and remain password protected. [Sponsor] will not have access to any identifying information from participants, and the participants' names and contact information will not be included in the electronic study database.

Specimens will be stored in a secure location at [site]. [Include the following if study includes establishment of a biorepository:] If the stored specimens are planned to be used for purposes other than this study, [institution(s)] will seek approval from the appropriate ethical committees. Stored specimens will be destroyed after a maximum of [number] years after study close.

## 10.6 Dissemination and publication policy

*Include a statement of the conditions under which the results of the clinical performance study will be published and describe plans for the dissemination of the study results.*

*Investigators should consider registration of the study in a public registry. More information is available at https://www.who.int/tools/clinical-trials-registry-platform.*

The data generated by this study will inform decisions regarding product development and commercialization of malaria RDTs. The data collected in this evaluation will [may] be used by the manufacturer to support the regulatory dossier[s] for the investigational product[s]. In addition, the study

will generate valuable data on the performance of this [these] novel test[s] in comparison to that of currently available comparator RDTs, enabling informed decision-making regarding recommendation of new highly sensitive point-of-care tools for malaria. Data will be shared with study partners and the test manufacturer. This may include de-identified, individual-level data.

All data will be published in the open medical literature with the identity of the participants protected. Authorship will be determined by mutual agreement and in line with the International Committee of Medical Journal Editors' authorship requirements, as described in the publication policy section of the contractual agreement.

Groups that supervise the study may access the results. This includes members of the ethics committee[s] of [list IRB(s)] and any other test auditor or regulator. Only de-identified data will be shared with groups outside of the study team.

# 11. Quality management, monitoring, safety, and reporting

## 11.1 Quality management

Quality management for this study will consist of quality control activities/monitoring, training, and use of SOPs, work instructions, tools, and templates.

The study will be conducted in accordance with the current approved protocol, ICH's GCP, relevant regulations, and SOPs.

## 11.2 Quality control/monitoring

Regular monitoring will be performed according to ICH GCP. Data will be evaluated for compliance with the protocol and for accuracy in relation to source documents. A monitoring report summarizing key indicators for study compliance will be generated every week. These indicators include but are not limited to the number of participants consented, the number of samples acquired, any deviations from study procedures, and corrective actions taken. Following written SOPs, study monitors will verify that the clinical study is conducted and data are generated, documented, and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

[Sponsor] and [site] will conduct necessary staff training on study procedures prior to initiating the study. Only research staff who have been trained in best practices for specimen collection and infection prevention will be involved in specimen collection. Only trained users who have been certified as proficient in the use of the study products will be responsible for conducting testing.

The study team will be supervised by the local study lead. [Sponsor] and [site] will hold regular study review calls to discuss data collection and data quality to date. In addition, a sponsor representative may conduct site monitoring visits as needed/feasible to ensure compliance with the protocol and relevant SOPs.

[Sponsor] will designate trained and qualified personnel to monitor the progress of this clinical study in accordance with study-specific SOPs. Prior to study start, a study training will be conducted to train staff

on the protocol, the completion of study documentation and data collection forms, the monitoring schedule, and all regulatory requirements. A [sponsor] representative may conduct remote (or in-person, if feasible) site monitoring visits as needed to ensure compliance with the protocol and relevant SOPs.

## 11.3 Quality assurance

The study site may be subject to a quality assurance visit. If so, the site will be contacted in advance to arrange a monitoring visit. The investigator and site staff will guarantee direct access to all study documents for quality assurance monitors.

## 11.4 Safety considerations

*Although studies of this nature are of minimal risk to participants, clinical protocols involving evaluation of IVDs should include safety considerations and reporting requirements. As per ISO 20916:2019, these should include definitions of adverse events, severe adverse events, adverse device effects, and device deficiencies; the reporting requirements, time frames, and processes associated with these events; a list of foreseeable events that could be anticipated in the study; and emergency contact details for reporting.*

We anticipate that this study poses minimal risk to participants, as it does not involve any medical intervention and blood draw volumes are within acceptable ranges. No data safety monitoring board will be used. Given that the only intervention undertaken in the study is capillary and venous blood collection and only the results from the standard of care test will be used for patient management, the probability of an adverse event (AE) or a serious adverse event (SAE) occurring to a study participant is extremely low. Nevertheless, safety and incident reporting is described below.

### 11.3.1 Adverse events

An **Adverse Event (AE)** is any untoward or unfavorable medical occurrence in a human subject—including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease—temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

An **Adverse Device Effect (ADE)** is an AE related to the use of an IVD medical device under investigation. This includes any event resulting from insufficient or inadequate instructions for use, installation, operation, or any malfunction of the IVD under investigation. This also includes any event resulting from use error or intentional misuse of the device.

A **Serious Adverse Event (SAE)** is any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- Results in death.
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly/birth defect.
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in

this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

A **Serious Adverse Device Effect (SADE)** is any ADE that has resulted in any of the consequences characteristic of a SAE.

Adverse events will be considered to be study related if the event follows a reasonable temporal sequence from a study-specific procedure and could readily have been produced by that study procedure.

Deaths, organ failure, and other complications related to a participant's underlying conditions (including the disease or problem that caused the participant to seek medical care) should **not** be considered study-related adverse events. **For the purpose of this study, only the collection of the venous and capillary blood samples is considered to be a study procedure.**

Any AEs that are unanticipated, serious, and related or possibly related to participation in the research, any SAEs, or any incidents that suggest that the research places participants or others at risk, including breach of confidentiality, will be promptly reported by the investigator or an appropriate designee to [sponsor] and the IRB[s] in accordance with the reporting requirements and required time frame of the IRB[s]. A complete written report will follow the initial notification. Other incidents will be reported in the annual continuing review report. There will be only passive monitoring of AEs that occur during the study visit period (from the time the participant signs consent until completion of sample testing). AEs that are spontaneously reported by participants during their visit will be documented.

The investigator will report any incidents that occur due to the medical devices used in the study in order for the sponsor and/or the manufacturer to notify appropriate regulatory authorities about relevant and required safety information relating to medical devices being used in this clinical study.

### 11.3.2 Device deficiencies

A **Device Deficiency** is defined as any inadequacy of a medical device with respect to its identity, quality, durability, reliability, usability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequacy in the information supplied by the manufacturer, including labeling.

Malaria RDTs are considered medical devices. In order to fulfill regulatory reporting obligations, the site will be responsible for the detection and documentation of any device deficiency, malfunction, or deterioration in the characteristics and/or performance of the device as well as any inadequacy in the labeling or the instructions for use, on a standardized form.

# 12. Ethical considerations

*The protocol should include a description of ethical considerations relating to the study. This should include not only information on how/from whom ethical approval will be obtained but should also include discussion of relevant ethical issues. It should also describe how the investigator(s) plan to obtain informed consent from the research participants (the informed consent process) and a consideration of the study's risks and benefits.*

## 12.1 Guiding principles

*In accordance with the "Statement of compliance" (page 9), the protocol should specify the ethical principles being followed in the study.*

The investigators will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the ICH GCP regulations and guidelines, whichever affords the greater protection to the subject. Additionally, the investigator assures that all activities of this protocol will be guided by the ethical principles of *The Belmont Report* and 45 CFR 46 and all of its subparts (A, B, C, and D). Investigators and study staff are trained and certified in GCPs and the protection of human subjects. Training in the principles of informed consent and in the study procedures for obtaining informed consent will be conducted before study initiation.

## 12.2 Informed consent

*Procedures for obtaining informed consent should be described in the protocol.*

Study team members trained in the principles of informed consent and human subjects protection will obtain written informed consent from all participants according to the procedures described in section 7 above.

## 12.3 Risk considerations and mitigation strategies

The RDT[s] under investigation is considered low risk to study participants because the test[s] is an IVD tool that requires a low volume of blood. Study procedures do not represent significant risks to the participants beyond those that are associated with a normal blood draw, such as pain, discomfort, feeling light-headed, fainting, and infection at the site of finger stick or venipuncture. The risks associated with blood draws will be mitigated through adherence to standard clinic procedures for infection control and through the use of research staff who have been trained in best practices for blood collection. The volume of blood drawn as part of the study procedures is within the safely limits recommended by WHO and other organizations for both adults and children.[21] All decisions regarding clinical care or malaria case management will follow standard procedures and will be made through referral to the local health care facilities.

The study staff are at risk for exposure to bloodborne pathogens in the course of their work. All study team members will adhere to standard procedures for infection control. Study staff exposed to bloodborne pathogens during the course of their study roles will follow their institutional guidelines for post-exposure prophylaxis.

There is a minimal risk that the lay providers/health care workers recruited for the usability study may feel compelled to participate in the study as part of their employment. They may feel as though the usability study is intended to assess their performance rather than the usability of the test[s]. We will mitigate these risks through the following measures: (1) Study staff will ask supervisors to explain that participation in the study is voluntary and will not affect employment in any way, and (2) consent procedures will be conducted in private to ensure confidentiality. During the consent procedure, participants will be informed that the aim of the study is to understand the user experience and the data will be used for purposes of product development only. The data will not be used to assess their competency or linked in any way to their job performance.

All efforts will be made to maintain confidentiality and data security. All study staff will participate in training for maintaining study confidentiality, including securing data on password-protected devices, keeping paper forms in locked cabinets/rooms, and maintaining all information collected by the study as confidential, not to be shared with individuals who are not part of the research team.

## 12.4 Benefits

Knowledge gained from this study may benefit society by providing information on the diagnostic accuracy of new and improved malaria RDTs. Data obtained from this study will be made available to the test manufacturer to support regulatory dossiers and ultimately to provide malaria-endemic countries with quality-assured malaria RDTs with improved analytical and clinical performance.

## 12.5 Risk-to-benefit rationale

Given the minimal risks associated with the study and the potential benefits to society, the benefits outweigh the risk. As with any clinical study, there is the possibility of unforeseen risks. If unforeseen risks are identified, all relevant parties, including the sponsor, site[s], IRB[s], and regulatory bodies will be provided with relevant information.

## 12.6 Study costs and compensation

Participants will receive [specify compensation amount, if applicable] for their time and participation in the study. No participant will be required to travel or incur any costs as part of their participation in this study.

## 12.7 Ethical review

The protocol, informed consent form, and recruitment materials will be submitted to [list overseeing IRB(s)] for written approval. The study will comply with any requirements required by the overseeing ethics committees.

### 12.7.1 Amendments

All amendments and modifications will be submitted to the IRB[s] listed in section 12.7 for review and approval. No changes in protocol conduct will be implemented until all approvals by the IRB[s] are obtained.

### 12.7.2 Continuing review reports

The Principal Investigator will be responsible for submitting the required continuing review report(s) and associated documents to the relevant IRB[s], allowing sufficient time for review and continuation documentation prior to the established continuing review date. A closeout report will be submitted upon completion of the study.

### 12.7.3 Reporting

*The protocol should describe procedures for reporting any deviation(s) from the original statistical plan, emergency contact details for reporting SAEs and SADEs, and notification requirements and time frames.*

Any deviation from the protocol that may have an impact on the safety or rights of the participant or the integrity of the study will be promptly reported to the appropriate IRB[s] within the required time frame from which the deviation is identified. All other deviations will be similarly reported to the appropriate IRBs in the annual continuing review report.

## 12.8 Care for injury

In the unlikely event of a research-related injury, the cost of treatment will be covered by the study, without the participant having to assume any expense.

## 12.9 Insurance statement

*Include a statement specifying the type of insurance that will be provided for participants, when applicable.*

[Include if applicable:] The sponsor has taken out insurance for the study in the event of a study-related injury.

# 13. Investigator responsibilities

*Alignment on roles and responsibilities of involved institutions is a critical component of any clinical study. To facilitate this, it may be useful to complete a roles and responsibilities matrix either as part of the protocol or the agreement between the sponsor and/or implementing research partner.*

The project partners involved in this research are [institution name] (study sponsor) and [institution name] (implementing research partner). Roles and responsibilities for each of the parties are listed below in Table 7. L=lead; A=assist.

**Table 7. Roles and responsibilities of project partners.**

| Task | [Name] | [Name] |
|---|---|---|
| Award oversight | | |
| Study design and protocol development | | |
| Development of data collection forms (case report forms) | | |
| Development of study database | | |
| Institutional review board submission | | |
| Regulatory submissions (as applicable) | | |
| Logistics arrangements | | |
| Procurement of study supplies | | |
| Training on the use of study assays | | |
| Implementation of the study according to the institutional review board–approved protocol (recruitment, consent, enrollment, and data collection) | | |
| Study monitoring | | |
| Data entry and cleaning | | |
| Data analysis and reporting | | |

# 14. Bibliography


1. Dhorda M, Ba EH, Kevin JK, et al. Towards harmonization of microscopy methods for malaria clinical research studies. *Malaria Journal*. 2020;19(1):324. doi:10.1186/s12936-020-03352-z
2. Cunningham J, Jones S, Gatton ML, et al. A review of the WHO malaria rapid diagnostic test product testing programme (2008–2018): performance, procurement and policy. *Malaria Journal*. 2019;18(1):387. doi:10.1186/s12936-019-3028-z
3. Aidoo M, Incardona S. Ten years of universal testing: how the rapid diagnostic test became a game changer for malaria case management and improved disease reporting. *American Journal of Tropical Medicine and Hygiene*. 2021;106(1):29–32. doi:10.4269/ajtmh.21-0643
4. World Health Organization. How malaria RDTs work. Accessed May 16, 2025. https://www.who.int/teams/global-malaria-programme/case-management/diagnosis/rapid-diagnostic-tests/how-malaria-rdts-work
5. Mouatcho JC, Goldring JPD. Malaria rapid diagnostic tests: challenges and prospects. *Journal of Medical Microbiology*. 2013;62(pt 10):1491–1505. doi:10.1099/jmm.0.052506-0
6. Li B, Sun Z, Li X, et al. Performance of pfHRP2 versus pLDH antigen rapid diagnostic tests for the detection of *Plasmodium falciparum*: a systematic review and meta-analysis. *Archives of Medical Science*. 2017;13(3):541–549. doi:10.5114/aoms.2017.67279
7. Ashley EA, Touabi M, Ahrer M, et al. Evaluation of three parasite lactate dehydrogenase-based rapid diagnostic tests for the diagnosis of falciparum and vivax malaria. *Malaria Journal*. 2009;8:241. doi:10.1186/1475-2875-8-241
8. Jimenez A, Rees-Channer RR, Perera R, et al. Analytical sensitivity of current best-in-class malaria rapid diagnostic tests. *Malaria Journal*. 2017;16(1):128. doi:10.1186/s12936-017-1780-5
9. Ding XC, Ade MP, Baird JK, et al. Defining the next generation of *Plasmodium vivax* diagnostic tests for control and elimination: target product profiles. *PLOS Neglected Tropical Diseases*. 2017;11(4):e0005516. doi:10.1371/journal.pntd.0005516
10. Gamboa D, Ho MF, Bendezu J, et al. A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack *pfhrp2* and *pfhrp3*: implications for malaria rapid diagnostic tests. *PLOS One*. 2010;5(1):e8091. doi:10.1371/journal.pone.0008091
11. Watson OJ, Tran TNA, Zupko RJ, et al. Global risk of selection and spread of *Plasmodium falciparum* histidine-rich protein 2 and 3 gene deletions. *medRxiv*. Preprint posted online January 1, 2024. doi:10.1101/2023.10.21.23297352
12. World Health Organization (WHO), Global Malaria Programme. *False-Negative RDT Results and Implications of New Reports of P. falciparum Histidine-Rich Protein 2/3 Gene Deletions*. WHO; 2017. https://www.who.int/docs/default-source/documents/publications/gmp/false-negative-rdt-results.pdf?sfvrsn=ec917b72_2
13. World Health Organization (WHO). *WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy*. WHO; 2010. https://www.who.int/publications/i/item/9789241599221

14. World Health Organization (WHO), United Nations Children's Fund/United Nations Development Programme/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). *Microscopy for the Detection, Identification and Quantification of Malaria Parasites on Stained Thick and Thin Blood Films in Research Settings (Version 1.0): Procedure: Methods Manual*. WHO on behalf of TDR; 2015. https://apps.who.int/iris/handle/10665/163782

15. World Health Organization (WHO). *Collection of Finger-Prick Blood and Preparation of Thick and Thin Blood Films: Malaria Microscopy Standard Operating Procedure – MM-SOP-05A*. WHO; 2016. https://www.who.int/publications/i/item/HTM-GMP-MM-SOP-05a

16. Rosen M, Dhorda M, Bell D, Gatton M. *Obare Method Calculator*. Version 1.0. WorldWide Antimalarial Resistance Network; 2015. https://www.wwarn.org/tools-resources/procedures/obare-method-calculator

17. Obare P, Ogutu B, Adams M, et al. Misclassification of Plasmodium infections by conventional microscopy and the impact of remedial training on the proficiency of laboratory technicians in species identification. *Malaria Journal*. 2013;12:113. doi:10.1186/1475-2875-12-113

18. Gonçalves L, Subtil A, de Oliveira MR, do Rosário V, Lee PW, Shaio MF. Bayesian Latent Class Models in malaria diagnosis. *PLOS One*. 2012;7(7):e40633. doi:10.1371/journal.pone.0040633

19. Das D, Dahal P, Dhorda M, et al. A systematic literature review of microscopy methods reported in malaria clinical trials. *American Journal of Tropical Medicine and Hygiene*. 2020;104(3):836–841. doi:10.4269/ajtmh.20-1219

20. World Health Organization (WHO). *Technical Specifications Series for Submission to WHO Prequalification – Diagnostic Assessment. TSS-3: Malaria Rapid Diagnostic Tests*. Licence: CC BY-NC-SA 3.0 IGO. WHO; 2017. https://apps.who.int/iris/bitstream/handle/10665/255038/9789241512275-eng.pdf

21. Howie SRC. Blood sample volumes in child health research: review of safe limits. *Bulletin of the World Health Organization*. 2011;89(1):46–53. doi:10.2471/BLT.10.080010.