TMDA/DMD/MDA/PROT/006
Revision: 00

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

TANZANIA MEDICINES AND MEDICAL DEVICES AUTHORITY

PROTOCOL FOR PERFORMANCE LABORATORY EVALUATION OF MALARIA *PLASMODIUM FALCIPARUM* (PF) AND MALARIA PF/PAN ANTIGEN RAPID DIAGNOSTIC TESTS

FIRST EDITION

OCTOBER, 2025

TMDA Headquarters, Plot No. 56/1, Block E, Kisasa B Centre, Hombolo Road, P. O. Box 1253, Dodoma - Tanzania, Tel: +255 (26) 2961989/2061990/ +255(22) 2450512/2450751/2452108, Email: info@tmda.go.tz, Website: www.tmda.go.tz, Toll free: 08001100834

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

This first edition of the protocol intends to establish a well-documented procedure for conducting performance evaluation of in vitro diagnostic tests for detection of Malaria Antigens. It is my hope that the information included in this protocol will assist manufactures and/or applicants to conduct performance evaluation studies in Tanzania Mainland and subsequently expedite the verification process of the results by the Authority.

I would like to take this opportunity to express my sincere gratitude to the Tanzania Medicines and Medical Devices Authority (TMDA) staff and all experts who dedicated their valuable time and experience to the development of these guidelines.

Our acknowledgements particularly go to Ms. Rehema Mariki, Dr. Goodluck Gotora, Mr. Christian Kapinga, Ms. Edina Zebedayo, Mr. Edinanth Gareba, Mr. Octavian Aron Ngoda, Mr. James Tanguye, Ms. Emmanuela Mkalawa, and Ms. Adelina G. Gadiye from Muhimbili National Hospital (MNH).

Moreover, I wish to gratefully acknowledge the outstanding technical contribution provided by Prof. Willy Urassa, a World Health Organization (WHO) consultant, since the early stages of the protocol development."

Finally, I would also like to thank the International Medical Devices Regulators Forum (IMDRF), the World Health Organization (WHO) as well as Medical Device Coordination Group (MDCG) established by European Union (EU) for making their guidelines available for referencing.

The proper use and implementation of this protocol will ensure that the detection of Malaria Antigens which have been designated to be of public health importance will perform clinically as claimed by manufacturers.

Dr. Kissa W. Mwamwitwa
Director for Medical Devices and Diagnostics Control

FOREWORD

Malaria continues to be a major public health challenge in low- and middle-income countries (LMICs) and in particular Tanzania, where timely and accurate diagnosis is essential to reducing the disease burden. In response to this urgent need, the Tanzania Medicines and Medical Devices Authority (TMDA has created a detailed protocol for evaluating the performance of Malaria Plasmodium falciparum (Pf) and Malaria Pf/Pan antigen rapid diagnostic tests (mRDTs).

This protocol provides a structured approach to evaluating the sensitivity, specificity, and operational performance of mRDTs, ensuring that they meet stringent regulatory requirements. By incorporating internationally recognized standards such as ISO 15189, ISO 17025, and WHO guidelines, it serves as a robust framework for the consistent assessment of diagnostic products intended for use in Tanzania. Furthermore, this protocol addresses key aspects such as lot-to-lot variability, inter-reader variability, and the usability of tests in various healthcare settings, ensuring the generation of high-quality data that informs regulatory decisions.

Manufacturers, applicants, and evaluating laboratories are expected to follow this protocol rigorously to streamline and expedite the approval process for mRDTs in Tanzania. The information gathered from performance evaluations conducted using this protocol will help safeguard public health by enabling the availability of reliable, high-performing diagnostic tools for malaria management.

TMDA recognizes the collaborative effort required to enhance diagnostic capacity, and this protocol stands as a demonstration to our ongoing commitment to harmonizing regulatory processes while ensuring the safety and effectiveness of diagnostics. Stakeholders are encouraged to share feedback for the continuous improvement of this protocol, as we work together to tackle malaria with the most effective diagnostic solutions available.

Dr. Adam M. Fimbo
DIRECTOR GENERAL

ABBREVIATIONS

EIA	-	Enzyme Immunoassay
IFU	-	Instructions for use
mRDT	-	Malaria Rapid Diagnostic Test
Pan	-	Other *plasmodium species*
Pf	-	*Plasmodium falciparum*
PI	-	Principal Investigator
QC	-	Quality Control
qPCR	-	Quantitative Polymerase Chain Reaction
TMDA	-	Tanzania Medicines and Medical Devices Authority

DEFINITION OF TERMS

Authority
Means the Tanzania Medicines and Medical Devices Authority or the acronym “TMDA” established under section 4(1) of the Act.

Applicant
Means any person or institution or company that applies formally for performance evaluation of IVD in Tanzania.

Clinical Performance
Means ability of an IVD to yield results that are correlated with a particular clinical condition/ physiological state in accordance to target population and intended use.

Conformity Assessment
Means the systematic examination of evidence generated and procedures undertaken by the manufacturer, under requirements established by the Authority, to determine that an IVD is safe and performs as intended by the manufacturer and, therefore, conforms to the Essential Principles of Safety and Performance of Medical Devices.

Diagnostic Sensitivity
Means the ability of a device to identify the presence of a target marker associated with a particular disease or condition.

Diagnostic Specificity
Means the ability of a device to recognize the absence of a target marker associated with a particular disease or condition.

Ethics Committee (EC)
Means an independent body composed of members with expertise in both scientific and non-scientific arenas which functions to ensure the protection of human rights and the well-being of research subjects based on six basic principles of autonomy, justice, beneficence, no maleficence, confidentiality and honesty.

Error rate
Means a measure of the degree of prediction error of a model made with respect to the true model.

In Vitro Diagnostics Devices
Means a device whether used alone or in combination, intended by the manufacturer for the in vitro examination of specimens derived from the human body and animals principally to provide information for diagnostic, monitoring or compatibility purposes. IVD include reagents, calibrators, control materials, specimen receptacles, software, and related instruments or apparatus or other articles and are used for example for the following test purposes: diagnosis, aid to diagnosis, screening, monitoring, predisposition, prognosis, prediction and determination of physiological status.

Manufacturer
Means any person or a firm that is engaged in the manufacture of IVDs.

Negative likelihood ratio (LR-)
Means a probability that a person with the disease tested negative divided by the probability that a person without the disease tested negative.

Negative predictive value
Means the ratio of subjects truly diagnosed as negative to all those who had negative test results (including patients who were incorrectly diagnosed as healthy).

Performance evaluation
Means the assessment and analysis of data to establish or verify the performance (analytical performance and where applicable, clinical performance) of an IVD.

Positive predictive value
Means the ratio of patients truly diagnosed as positive to all those who had positive test results (including healthy subjects who were incorrectly diagnosed as patient).

Positive likelihood ratio (LR+)
Means probability that a positive test would be expected in a patient divided by the probability that a positive test would be expected in a patient without a disease.

1 Introduction

Malaria remains among the major causes of morbidity and mortality in Tanzania, necessitating reliable diagnostic methods to support timely and accurate treatment. Malaria rapid diagnostic tests (mRDTs) have become an essential component of malaria management, especially in settings with limited access to laboratory infrastructure. To ensure the effectiveness of mRDTs in detecting Plasmodium falciparum (Pf) and other malaria-causing species, the Tanzania Medicines and Medical Devices Authority (TMDA) has developed this performance evaluation protocol.

This protocol is designed to provide a comprehensive framework for assessing the clinical performance, sensitivity, specificity, and operational characteristics of mRDTs. By evaluating these diagnostic tools in comparison with established reference methods such as microscopy and polymerase chain reaction (PCR) the protocol ensures that only products meeting the required performance criteria are authorized for use in Tanzania. The evaluation will use well-characterized blood specimen panels drawn from different regions in Tanzania, thereby reflecting the country's genetic and epidemiological diversity.

In addition to performance criteria, the protocol addresses lot-to-lot variability, inter-reader variability, and usability under real-world conditions, including variations in environmental factors. By following this protocol, manufacturers and applicants will generate essential data that supports regulatory decisions while safeguarding public health through the availability of reliable diagnostic tools in Tanzania's fight against malaria.

This protocol should be read together with the current TMDA Guidelines for Conducting Performance Evaluation of In-vitro Diagnostic Devices submitted for marketing authorization which among other things provides for format of clinical performance study report based on the class of the IVD medical device.

2 Study objectives.

2.1 Overall objective

The overall objective is to evaluate and compare the accuracy of currently available simple rapid tests for detection of Malaria Pf and Malaria Pf/Pan antigens against established performance criteria.

2.2 Specific objectives

The specific objectives of the performance evaluation are:

1. To determine the sensitivity and specificity of currently commercially available Malaria Pf and Malaria Pf/Pan Antigen RDTs as compared to a reference algorithm consisting of Microscopy, and Polymerase Chain Reaction (PCR).
2. To assess lot-to-lot variation
3. To assess inter-reader variability (for subjectively read assays)
4. To describe and assess the operational characteristics and ease of use of mRDT for antigens detection.

3 Study implementation.

3.1 Performance evaluation laboratory

The performance evaluation laboratory shall hold one of the following certifications for quality management within the laboratory: ISO17025 *General Requirements for the competence of testing and calibration laboratories*, ISO15189 *Medical laboratories: Requirements for quality and competence* or equivalent.

The Head of the Laboratory will act as the Principal Investigator (PI) for the work performed by the laboratory.

3.2 Training, performance evaluation and supervision

The following issues are key to minimizing error and maximizing the value of this performance evaluation:

- The PI will be responsible for training the laboratory professionals on the details of the evaluation protocol and on the performance of each assay undergoing evaluation.
- Only those personnel who have received specific training for a particular assay evaluation will be employed.
- Accurate record keeping is crucial to the success of the evaluation and the PI will be responsible for ensuring that all data required for the evaluation are recorded on the agreed data collection sheets and are accurate and up to date.
- It is important to plan work in advance and follow standard operating procedures as prepared and controlled by the laboratory.
- To reduce the risk of adding an incorrect specimen to a test device/well, before starting the test run, the operator will prepare worksheets and label all tubes, dilution vessels, test devices or plates with the specimen's unique number.
- Because objective, machine-generated, permanent results for simple/rapid diagnostic tests are not feasible, it is essential that the PI emphasizes to the operator performing the tests the need for accurate recording of results and recordkeeping.

- To minimize the risk of error, it is recommended that the results are read and recorded independently by three trained staff members.
- To allow immediate correction of erroneous recording of results (rather than differences in visual interpretation), the PI or designee should assess the results as soon as possible to allow her/him to return to the original test device to investigate apparently discordant readings.
- For the performance evaluations performed, at least one representative result from both Malaria positive and negative specimens will also be recorded by taking electronic images. Unexpected test results will also be digitally.

3.3 Storage of assays

All reagents must be stored as indicated in the instructions for use. Some assays may not need refrigeration. If refrigerated storage space is inadequate to store the entire test kit, they may be divided so that labile reagents can be refrigerated separately from the non-labile supplies. Calibrated thermometers are placed at each location where reagents and specimens are stored, i.e. ambient, refrigerator and freezer. Temperatures are recorded daily on the laboratory temperature logs. The lot numbers of the test kits received/used, and their expiry dates are recorded on the individual run worksheets.

Two separate production lots (with different lot numbers and different expiry dates) will be requested for evaluation, according to the following definition of a lot: “The amount of material that is uniform in its properties and has been produced in one process or series of processes. The material can be either starting material, intermediate material or finished product.” Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents. Furthermore, lots must be sourced from a representative production run and not produced especially for the purpose of this evaluation. TMDA will verify this information before the product assessment has been finalized.

4 Specimens

4.1 Clinical performance specimen panel

A panel of 1500 well-characterized venous or capillary whole blood clinical specimen, including 500 (450 *Plasmodium falciparum* and 50 other *plasmodium* species) Malaria antigen positive specimens and 1000 Malaria antigen negative specimens, will be used for this evaluation.

4.2 Patient Eligibility

The study population will be drawn from subjects that meet the following enrolment criteria.

Inclusion Criteria

i. Subject is age 2-80 years old; male or female.
ii. Subject is experiencing fever or chills for one or more days.
iii. Subject is willing and able to participate in this clinical study.

Exclusion Criteria

i. Study investigator deems the subject's participation to be unsafe due to an underlying medical condition.
ii. Subject is under antimalarial treatment.
iii. Specimen volume is inadequate.
iv. Specimen collected via an unacceptable method or specimen has expired or deteriorated.
v. Specimen mistakenly enrolled by operator and/or with unconvincing results and/or cannot be traced.

For point iii to v patients will still be managed at the health care facilities, according to the National treatment guideline, since only venous blood will be excluded for detailed analysis (qPCR)

4.3 Sample Collection

4.3.1 Capillary blood

Subject recruitment in the field will be based on the inclusion/exclusion criteria stated in and will ensure each subject reads or clinician read the Inform Consent Form (ICF) for patients and make them understands before signing the ICF (Annex 2). A unique identification number will be assigned to each subject and a sample track number for every finger stick and every venous whole blood specimen. Subject Screening and Enrolment Log will be filled and completed at the enrolment site (health care facilities).

After signing a consent form, a fingerstick whole blood specimen will be collected from each subject with an inverted cup provided in the kit. After that, a fingerstick whole blood specimen will be collected, prepared and tested on the Malaria P.f and Pf/Pan Rapid Test cassette. The result will be recorded on the patient information sheet. A photo will be taken of the result as the original data.

From the same subject, a health professional, a trained nurse, laboratory technician or clinician will collect 5mL venous whole blood (sample B track number) in a collection tube with K_2EDTA, citrate or heparin for laboratory testing (venous blood testing on Malaria P.f and Pf/Pan Rapid Test Cassette, PCR testing and microscope testing.) Venous blood will be transported from the health care facilities in the field in a cooler box to the clinical laboratory

All test results (fingerstick results and laboratory testing results) will be recorded on the test record. The evaluation of the test kits will be done in the in the field (dispensaries and health care centers) and in the clinical research laboratory. To evaluate usability of the two test kits, environmental temperature and humidity as well as site conditions, (indoor, outdoor, weather- lighting) will be recorded during testing of Malaria *P.f* and *Pf/Pan* Rapid Test Cassette, in the health care facilities in the field and clinical laboratory. The results of all tests will then be entered into the database for data analysis. Any repeat testing will be noted on the record.

4.3.2 Venous Whole Blood

Venous blood will be collected according to the SOP and sample collection manual into a collection tube (containing K_2EDTA, citrate or heparin) Testing will be performed as soon as possible after specimen collection. If specimens are not tested immediately, they will be stored at 2-8°C for up to 3 days. Bring specimens to room temperature prior to use. Store and transport the venous whole blood collection tube in a temperature-controlled box (2-8℃) to the laboratory as soon as possible (within three days after collection). Do not freeze the samples for testing mRDTs or preparation of blood slides. To maintain the quality of RNA and DNA, the EDTA blood will be frozen at minus 80℃ until tested for qPCR.

Patients seeking medical care at health care facilities will be enrolled. Patients will be informed about the performance evaluation objectives by the clinicians or nurse and those meeting enrolment criteria including the signing of informed consent will be enrolled. A finger-prick whole blood specimen and a 5 ml of venepuncture whole blood specimen will be collected from each subject. In the field, the finger prick whole blood specimen will be immediately tested with the Malaria P.f and P.f/Pan Ag Rapid Tests Cassette. In the laboratory, the venepuncture whole blood specimen will be tested with the Malaria P.f and P.f/Pan Ag Rapid Test Cassette.

The clinical truth of the specimen will be confirmed using the venepuncture whole blood specimen using PCR method and microscopy will be done at the clinical laboratory. Malaria will be defined as ≥1 parasite seen in any blood sample.

5 Laboratory testing

5.1 Malaria Parasite detection by Rapid Diagnostic Test (RDT)

Finger prick samples of 5 mL blood will be collected by plastic dropper at enrolment site or from EDTA tube for examining malaria parasites by RDT; both test and standard RDT. Immediately test the fingerstick whole blood specimen with the Malaria P.f Rapid Test Cassette. The results will be recorded depending on the instructions for use of each kit. Results will be declared positive if lines observed on P. falciparum or only Pf/Pan bands line and negative if neither line observed on P. falciparum nor Pf/Pan band lines. Reference to the test kit manual will be used.

5.2 Malaria Parasite detection by Microscope

Venous whole blood in EDTA will be used to prepare blood smears which will be stained for 15 minutes using 10% Giemsa stain and screened microscopically for asexual parasites and gametocytes. Slides will be declared negative if no parasites are observed in 100 microscopic fields. Asexual parasites and gametocytes will be counted against 200 and 500 white blood cells, respectively. If there is discrepancy between mRDT and microscopy results slides will be read twice for confirmation. Quality control for positive and negative slides will be done on daily basis.

5.3 Parasite detection by Polymerase Chain Reaction (PCR)

Venous blood 5mls sample will be collected in the EDTA tube stored at -80°C to maintain the quality of RNA and DNA until further extraction. Genetic material (DNA and RNA) will be extracted by the Boom method that is based on the lysing and nuclease-inactivating

properties of guanidinium thiocyanate together with the nucleic acid-binding properties of silica particles. The 18s QT-NASBA detects total parasites and gametocytes at a detection limit of 10 parasites/mL; the Pfs25 QT-NASBA is gametocyte specific and has a detection limit of 20-100 gametocytes/ml. Parasite detection by QT-NASBA will be done on a NucliSens EasyQ analyser (bioMérieux, Boxtel, The Netherlands).

5.4 Patient Data Collection and Retention

The patient data records will use a universally printed form. The test records shall be true, standardized and complete. The patient data records should be written with a blue or black writing pen or signature pen. In order to protect the privacy of the sample provider, the name of the sample provider (patient) shall not appear in the record, and shall be replaced by the unique patient number, or track number or letter initials. The patient data records shall not be modified or altered at will. If a revision need to be made, a slash shall be drawn through the revision (not completely blacked out) to ensure that the record before the revision can be identified. The modification should be signed by the revision person, indicating the revision time/date and reason. The test records shall not be copied, photographed or photocopied in any form to other person without the unanimous permission of the applicant and the research supervisor of the test site.

5.5 Review of the instructions for use

Each product under evaluation is used strictly in accordance with the instructions for use (IFU) issued by the manufacturer. The laboratory will send a hard or electronic copy of the IFU to TMDA upon delivery of the test kits and prior to the commencement of the testing of the blood samples. The IFU must be reviewed against the IFU submitted to TMDA as part of the dossier assessment for market authorization procedure. If the IFU has been updated since dossier submission, a letter from the manufacturer detailing changes made must be sent to TMDA prior to the evaluation commencing.

5.6 Clinical performance panel

The specimens are tested such that approximately one half of the specimen panel will be run with the one lot and the other half of the panel with the other lot. The malaria infection status will be based on combined results of microscopy and PCR in a **blinded manner**.

Specimens with invalid result should be retested in singular on the same lot.

Specimens which are found to be indeterminate by the criteria stated in the instructions for use should be retested in duplicate on the same lot number of assay and singular on the other lot. In the case that the testing result cannot be resolved after all testing, the specimen is to be called indeterminate.

Specimens with results discrepant from the reference results are retested in duplicate using the same lot number by the same operator. The results that occur two out of three times are recorded as the test results. If the result is again discrepant, the specimen is retested on a second lot number, if available. If the result on the second lot is concordant with the reference result, no further testing is required. In all cases of repeat testing, all results (initial and repeat testing) should be recorded.

5.7 Lot-to-lot variation panel

Lot-to-lot variation is assessed by testing the same ten dilution series on two separate production lots of the assay under evaluation in the same testing session.

5.8 Interpretation of results

The interpretation of results for each assay under evaluation is made strictly according to the manufacturers' instructions within the IFU. Invalid test results are recorded on the data collection sheets including where the control line does not appear or in any other way the test result is invalid as defined by the IFU. For test results that are indeterminate according to the IFU, the results are recorded on data collection sheets.

Visual interpretation of results of subjectively read assays is made independently by three readers (without the knowledge of the other two sets of results and blinded to the reference result for the specimen) and entered onto the data collection sheets. These results are compared by the operator carrying out the assay so that any mistakes may be identified and rectified immediately. Should recording errors be identified, both the original and corrected result are recorded and initialed by the reader. When the three readers interpret the results differently from each other (i.e. reactive/non-reactive), the consensus is recorded as that interpretation which occurs two out of three times. In cases where all three interpretations are different, the result is recorded as indeterminate.

5.9 Recording test results

All test results are recorded on standardized test result worksheets and then entered in a Microsoft Excel spreadsheet for further data analysis.

For subjectively read assays such as rapid diagnostic tests the intensity of band/line/spot is additionally entered into the data collection sheet. The intensity rating system reads as described in Table 2.

Table 1. Result legend for subjectively read assays.

Scoring index	Intensity reading scale
0	Non-reactive
1	Very Weak
2	Weak
3	Medium to Strong Reactivity
7	Invalid

6 Quality control and interpretation of test results

6.1 Test kit controls.

Manufacturer/Applicant supplied positive and negative test kit controls will be run as indicated in the IFU for all test formats included in each test run for PCR and at the commencement of each testing session for rapid diagnostic tests and other formats. Where positive and negative test kit controls are not supplied by the manufacturer/applicant, as will

be the case for many rapid diagnostic tests, the external quality control specimen will act at the control specimen.

6.2 Internal control lines for rapid diagnostic tests

Generally, rapid diagnostic tests contain a control band, line to determine migration of the reagents, or the sample has occurred. Most control bands/lines will become visible with the addition of dilution buffer. However, some rapid diagnostic tests will contain a control band/line that becomes visible with addition of specimen. It is imperative that the exact nature of the control band/line is ascertained and included in the report. An experiment is performed to verify this point, if not explicitly mentioned in the IFU.

6.3 External quality control specimen

The laboratory will supply an external quality control (QC) specimen which is tested in singular at the beginning of each test session for rapid diagnostic tests. The QC specimen represents a weakly reactive Malaria positive sample. The QC specimen is prepared by the laboratory or acquired commercially, depending on the assay under evaluation.

6.4 Competency panels

User competency must be established for each assay by each operator before the evaluation commences.

6.5 Limits of acceptability

All results on test kits controls and QC specimens are entered on the data collection sheets. Should the test kit controls or the QC specimen not give results within the expected ranges, evaluation testing on that assay is suspended until the cause has been identified and a satisfactory solution identified. Such problems must be communicated immediately to TMDA and recorded on the data sheets. The PI is responsible for carefully checking all data entry forms for legibility, accuracy and completeness.

7 Analysis of data

7.1 Invalid devices

The number of invalid devices (for rapid diagnostic test) is recorded as the number of invalid test devices as a percentage of the total number of devices used for the evaluation testing with clinical specimens (excluding, lot to lot variation panels).

Invalid results may mean invalid test results as defined by the instructions for use such as where the control line/band does not appear or invalid due to obviously defective test device or defective transfer pipette.

7.2 Inter-reader variability

The inter-reader variability is Calculated when test results must be read without any objective reading instruments i.e. rapid diagnostic tests. Three persons independently interpret each test result. The inter-reader variability is expressed as the percentage of specimens for which initial test results are differently interpreted (i.e. reactive or non-reactive or indeterminate, if applicable) by the independent readers for the clinical specimens.

8 Clinical performance characteristics

The following strategies are used to calculate the clinical performance characteristics by comparing the results of the assay under evaluation and reference testing results on the clinical specimen panel.

Table 2. 2 x 2 table for calculation of performance characteristics

	Reference testing results (Microscopy and PCR)			
Results of assay under evaluation		Malaria antigen positive	Malaria antigen negative	Total
	Reactive	a (true positives)	b (false positives)	a + b
	Non-reactive	c (false negatives)	d (true negatives)	c + d
	Total	a + c	b + d	a+b+c+d

8.1 Sensitivity

Sensitivity is the ability of the assay under evaluation to detect correctly specimens that contain the analyte (reference results positive). Thus, sensitivity is the number of true positive specimens identified by the assay under evaluation as positive (a), divided by the number of specimens identified by the reference assays as positive (a+c), expressed as a percentage.

$$\text{Sensitivity} = \frac{a}{a+c} x100\%$$

8.2 Specificity

Specificity is the ability of the assay under evaluation to detect correctly specimens that do not contain the analyte (reference results negative). Thus, specificity is the number of true negative specimens identified by the assay under evaluation as negative (d), divided by the number of specimens identified by the reference assays as negative (b+d), expressed as a percentage.

$$\text{Specificity} = \frac{d}{b+d} x100\%$$

8.3 Confidence intervals

The exact 95% confidence intervals for binomial proportions are calculated for both sensitivity and specificity.

8.4 Initial and final sensitivity and specificity

The initial sensitivity and specificity are calculated based on the initial results obtained for the assay under evaluation (except for invalid results, for which the result of repeated testing is used). If the initial result is indeterminate, then the specimen is excluded from this analysis. The proportion of initial indeterminate results is reported.

The final sensitivity and specificity values are calculated taking into consideration the repeat

testing performed on the same lot and further testing second lot of the assay under evaluation, if applicable (i.e. for specimens with initial indeterminate or discrepant results).

9 Analytical performance characteristics

9.1 Results from lot-to-lot variation panel

The results of the lot-to-lot panel for the two production lots are compared and a variation of +/- 1 dilution series is considered acceptable. The number of series with acceptable and non-acceptable variation is reported.

10 Laboratory professional's appraisal

The technical aspects of the assay under evaluation are assessed by the Laboratory professional(s) who performed the testing. These assessments, along with other selected assay characteristics, contribute to an overall appraisal of each assay's suitability for use in small laboratories. To enable comparison between assays, a scoring system is used to rate specified operational characteristics (Annex 1).

11 Report preparation and dissemination.

The preliminary data analysis and drafting of the report will be carried out by the evaluating laboratory according to pre-defined report templates.

The draft report will be sent to TMDA.

12 Acceptance criteria

The following criteria will be used to assess the assay under evaluation. Other parameters included in this evaluation are provided for information but are not used as pass/fail criteria for this assessment.

Table 3. Minimum acceptable performance for Malaria antigen serology assays in the TMDA prequalification performance evaluation

Parameter	Rapid diagnostic tests
Initial sensitivity estimate	≥ 95%
Final specificity estimate	≥ 95%
Inter-reader variability	≤5%
Invalid rate	<5%

13 Materials and supplies

The applicant of products will provide the products and any equipment necessary for the evaluation free of charge.

Table 4. Number of tests required to perform this evaluation.

	Lot A	Lot B
Clinical panel	750	750
Lot-to-lot variation	100	100
Total		
Total + 20% for controls and repeats (for antigen detecting assays)		

14 Roles and responsibilities

14.1 Responsibilities of the Evaluating Laboratory

i. Ensure availability of Malaria specimen, lot-to-lot variation panel;
ii. Conducting the performance evaluation in accordance with internationally recognized best practice;
iii. Preparation of QC specimens and proficiency panels;
iv. Preparation of draft report on laboratory evaluation;
v. Advising TMDA on operational characteristics of assays evaluated.

All source data, data analysis records and all correspondence are retained and archived for a period of at least ten years.

14.2 Responsibilities of TMDA

i. Technical advice to the PI;
ii. Technical and administrative management of the laboratory evaluation;
iii. Verification of the draft report, seeking of clarification from the manufacturer if any
iv. Preparation and dissemination of the final report;
v. Formal contacts with authorized contacts of the manufacturers.

15 Administrative, Ethical and Regulatory considerations

The Institutional Review Board and the Medical Research Coordinating Committee of National Institute for Medical Research will approve the protocol before its implementation to obtain Ethical Clearance Certificate for conducting RDT validation in Tanzania.

Participation in this study is voluntary and no study procedures will be initiated prior to written and signed/thumb printed ICF. The decision of not participating in the study will not affect the quality of care the participant is entitled from the clinic staff. The informed consent will be translated to the local language (Kiswahili) which is easily understandable by the local community where the study will be conducted. Informed consent will be obtained from all study participants.

The Laboratory Director and their representatives will keep the study protocol, documentation data, and all other information generated, in strict confidentiality. The Laboratory Director will not release any information concerning the study or the data to any unauthorized third party without prior written approval.

No donor names, medical record numbers, or other personal identifying information will be used as part of the evaluation. Data collection forms will not contain confidential information linking the outcome result to a patient. All procedures and tests performed during the evaluation will be conducted according to Good Clinical Practices (GCP) and Good Laboratory Practices (GLP). All testing must be performed according to the approved protocol. A copy of the IRB approval letter shall be obtained and filed in the Investigator Site File.

16 References

Armitage P, Berry G, Matthews, JNS, Statistical Methods in Medical Research, 4th Edition. Blackwell Scientific Publications, Oxford, 2002

Kirkwood B, Stern J, Essential Medical Statistics 2nd edition, Blackwell Science Ltd. 2003

WHO. Guidance on regulations for the transport of infectious substances 2021-2022. https://iris.who.int/bitstream/handle/10665/339825/9789240019720-eng.pdf?sequence=1

WHO. Guidance for procurement of in vitro diagnostics and related laboratory items and equipment.https://iris.who.int/bitstream/handle/10665/255577/9789241512558 eng.pdf?sequence=1

EN 13612:2002 Performance evaluation of in vitro diagnostic medical devices

ISO 17025 General requirements for the competence of testing and calibration laboratories

ISO15189 Medical laboratories — Requirements for quality and competence

17 Annex 1: Operational characteristics and ease of use

This assay requires/does not require laboratory equipment and can/cannot be performed in laboratories with limited facilities or in non-laboratory settings. *If applicable, add specifics on why it cannot be used in laboratories with limited facilities: e.g.* The instrument requires a stable source of electricity and significant physical space. Furthermore, training and implementation of good laboratory practice is essential to obtaining accurate results. *If technical support was needed during evaluation:* Adequate technical support from manufacturer or representative is critical.

The assay was found easy to use / not easy to use by the operators performing the evaluation. *If applicable, add specific information provided in comments in the ease-of-use table.*

For RDTs (and ELISA)

Key operational characteristics	
Number of steps*	X steps in total X steps with precision pipetting *(only for serum/plasma)*
Time to result	X minutes
Endpoint stability (interval)	X minutes (the test can be read between xx and xx minutes after addition of specimen/diluent)
Internal QC	Yes/no, *insert brief description.[[The test has an internal control line. The presence of the control line indicates that migration of liquid has occurred; however, it does not guarantee that the correct specimen type or volume was added or that the test procedure was followed correctly.]*

** Definition: each action required to obtain a result (excluding specimen collection, device preparation – opening the pouch), e.g. for RDTs: add specimen, add buffer (2 steps).*

For instrument-based assays

Key operational characteristics	
Number of steps for one specimen*	X steps in total X steps with precision pipetting
Number of steps for instrument management**	X steps per run/day
Time to result for one test/run	X minutes
Operator hands-on time for one test/run	X minutes
Level of automation	
Quality controls	QC are/are not provided by the manufacturer and should be purchased separately. *Add information*

	on type of QC (eg. high positive, low positive, negative)
Operating temperature	xx- xx °C, any comments on temperature stability of conducting the test.
Result display and connectivity	Results are displayed on the instrument / connected computer. They may be printed using a standard/specific printer. The results can be exported to the laboratory information system and other health information systems.
Power sources	Main power / Battery / Solar power The use of a UPS is recommended, as stable electricity is required
Biosafety *(outside of infectious specimen handling)*	Operators reported no biosafety concerns for the user. *Add information if applicable*
Waste	The volume of liquid was is approx. xx per test/run. The volume of solid waste is approx. xx per test/ run. Waste disposal requires / does not require specific measures in addition to usual laboratory biohazard waste disposal procedures. *Add information if applicable.*
Calibration	Calibrators are/are not provided by the manufacturer and should be purchased separately. *Add frequency of calibration recommended.*
Maintenance	Daily / Weekly / Monthly / Yearly / No maintenance is required.
Other specific requirements	*If applicable (eg. space requirements, weight to surface area ratio, installation by manufacturer, ..)*

18 Annex 2: Patient Information and Consent form (English)

Purpose of the performance evaluation study

In order to diagnose clinical malaria precisely, we need to do laboratory tests. The new malaria rapid tests take 15-20 minutes to diagnose malaria, but we don't know if they are accurate or reliable. The main purpose of this performance evaluation study is to evaluate the Malaria *P.f* and *Pf/Pan* Rapid Test Cassette manufactured by <insert Name> Co., LTD for Tanzania Medicines and Devices Authority. Malaria Pf Ag Rapid Test is used for the detection of Plasmodium falciparum antigens and Malaria P.f/Pan Ag Rapid Test are intended for qualitative detection of Plasmodium falciparum, P. vivax, P. ovale, P. malariae antigens in whole blood specimens only.

We would like to compare the result of these rapid tests with the results of laboratory-based tests (microscopy and PCR) to see if they sensitive and specific as laboratory tests.

Procedures to be followed.

If you agree to participate in this performance evaluation study, you will sign inform consent and assigned a unique number. We will prick your finger and take about 300µl of blood (about a teaspoon) and collect 5 mL from the vein. Your name will not appear on any specimens or study forms.

Voluntary participation

A decision not to participate or withdraw from participation will not affect the care you will receive at the clinic in any way. Even if you do agree to become a study participant, you can decide to withdraw from study at any time (verbally).

Discomfort and risks.

You may feel some discomfort or have a bruising on your finger and arm where the blood was taken.

Benefits

There will be no immediate benefits in your participation in this performance evaluation study. When the study results become available and the rapid test results are acceptable in terms of accuracy, everyone who comes to the clinic may benefit from having this test available to diagnose malaria and receive the right treatment the same day.

Compensation

There will be no monetary compensation for this study, but routine medical consultation and appropriate referral services are available. However, we will reimburse your travel cost not exceed 5000 Shillings.

Confidentiality statement

The records concerning your participation are to be used only for the purpose of this performance evaluation study. Your name will not be used on any study forms or labels on laboratory specimens or in any report resulting from this study. At the beginning of the study, we will give you a study identification number and this number will be used on the forms and on the laboratory specimens. Any information obtained in connection with this study will be kept strictly confidential. Only members of the study team (doctors, nurses) will have access to information linking your name with your study number.

Who can I contact if I have questions or emergency.

If you have any questions, an emergency or you need clarification at any time before signing the consent form or during the study period, do not hesitate to ask the study team members that have provided you with this information. You can also contact the Principal Investigator or co – investigator or IRB member if you have any questions or need clarification related to this study or related to your participation.

1. Principal investigator: <insert name>, Phone: <insert number>,, Email: <insert e mail>,

2. IRB Contact: Member of IHI IRB: <insert name>, Phone: <insert number>, Email: <insert e mail>,

CERTIFICATION BY A VOLUNTEER (ADULT CONSENT)

I, ---------- (Volunteer Name and Surname)

I attest to have received explanation about the aim and procedures of this performance evaluation study entitled above. I attest to have been given enough time to read the information script above that describes details of the study procedures, including samples to be collected. I understand that confidentiality will be preserved. I understand that I am free to withdraw from the trial at any time without affecting the care I normally receive at the clinic. I agree to participate in this study as a volunteer subject and will be given a copy of this informed consent to keep.

I attest to have understood the aim, procedures, benefits and risks and that I was given sufficient time to ask questions related to the procedures, benefits and risk of my participation and I have received satisfactory answers. I understand that if I have further questions, I can contact the study team by telephone or mail using the contact information above.

I also agree that my blood samples and related information will be stored for up to three years and that my personal information will be kept in a secure and protected manner. At the end of this study in three years, all samples, as well as all accompanying information, will be destroyed.

I VOLUNTARILY AGREE TO PARTICIPATE in the study referenced above.

I consent to full participation in the survey and sample collection for this study.

I consent for sample collection of my child for this study and other malaria kits evaluation study and diagnosis of other cause of fever.

NAME OF PARTICIPANT (PRINT) First name Middle name Surname

Date: …../……/……(dd/mm/yyyy)

Time ……:…..(Hrs)

Signature_____.

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

NAME OF PERSON GIVING CONSENT EXPLANATION (PRINT) First name Middle name Surname

Date: …../……/……(dd/mm/yyyy)

Time ……:…..(Hrs)

Signature_____

IF PARTICIPANT IS ILLITERATE

THUMB PRINT (OR OTHER MARK) OF PARTICIPANT

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

NAME OF THE WITNESS (PRINT) First name Middle name Surname

Date: …../……/……(dd/mm/yyyy)

Time ……:…..(Hrs)

Signature _____