THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

TANZANIA MEDICINES AND MEDICAL DEVICES AUTHORITY

PUBLIC ASSESSMENT REPORT FOR MALARIA PF AND MALARIA PF/PAN ANTIGEN RAPID TEST WHO PREQUALIFICATION CLINICAL STUDY

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TMDA Headquarters, Plot No. 56/1, Block E, Kisasa B Centre, Swaswa Road, P. O. Box 1253, Dodoma – Tanzania, Telephone: +255 (26) 2961989/2061990/+255 (22) 2450512/2450751/2452108, Email: info@tmda.og.tz, Website: **www.tmda.go.tz**

Toll free: 0800110084

1. INTRODUCTION

Correct patient management and implementation of sustainable control strategies require accurate and rapid diagnosis of malaria parasites to reduce morbidity and mortality, and drug resistance development due to overuse of antimalarial without diagnosis. In 2010 WHO recommended the use of antibody-based Rapid diagnostic tests (RDTs) to detect malaria antigens which is the most widespread method for malaria diagnosis in malaria-endemic countries. RDTs are easy to use at the point of care with minimal infrastructure. Tanzania rolled out the use of mRDTs and witnessed a huge improvement in case management and the rational use of antimalarial drugs. Currently, there are several commercial brands of mRDTs registered by the Tanzania Medicines and Medical Devices Authority (TMDA) such as SD Bioline HRP2/ pLDH, Para Check HRP2/ pLDH, CareStart HRP2/ pLDH that detects P. falciparum and other malaria parasite species. The wide use of mRDTs in healthcare facilities has shown a decline of malaria prevalence up to 7.3% in children under 5 years old based on mRDTs results as reported by the Tanzania Malaria Indicator Survey in 2017. Despite the remarkable benefits of the RDTs, batch-to-batch variations, misinterpretation, limited sensitivity and specificity and instability of stored test kits in tropical climate offers some disadvantages.

WHO provided a guideline titled “TSS-3 Technical Specifications Series for submission to WHO Prequalification-Diagnostic Assessment: Malaria rapid diagnostic tests”. The major objective of the guideline is to provide standard technical guidance for the assessment of in vitro diagnostic medical devices (IVDs) to the manufacturers who are seeking WHO prequalification of IVDs, and RDTs for malaria diagnosis (WHO, 2017). Manufacturers shall submit evidence of the clinical performance of the intended devices showing the procedures used to ensure the devices have been correctly operated by the users, detected the target pathogens and their full indication for use. The clinical performance studies for malaria rapid diagnostic tests (mRDTs) must be conducted in low- and middle-income countries, where the devices are likely to be used by a diversity of knowledgeable and skills personnel. It should also cut across the population of users

like laboratory technicians, healthcare workers or trained lay personnel and population such as paediatrics, symptomatic patients and those with clinical indication.

1.1. Study Rationale

WHO provided a minimum performance requirement to the manufacturers to provide evidence of the clinical performance of the IVDs in the different settings, environments and intender users in malaria-endemic areas where the diagnostics are more likely to be used. Tanzania is a malaria-endemic area and has rolled out the use of malaria rapid diagnostics tests since 2010. Ifakara Health Institute (IHI) is one of the selected sites to conduct a prequalification assessment for two mRDTs products Malaria Pf and Malaria Pf/Pan Antigen Rapid tests manufactured by Zhejiang Orient Gene Biotech Co., LTD. IHI established a clinical trials unit at Bagamoyo (IHI-Bagamoyo) which amongst other activities is to perform an assessment of IVDs. IHI-Bagamoyo has strong track records in testing and validating IVDs for public health (IHI, 2016). Since 2004, the laboratory unit at IHI-Bagamoyo offered services within IHI and outside to other research institutions, hospitals, and universities across the globe with ISO accredited. Previous studies conducted at IHI aiming to validate mRDTs with high technology (Hofmann et al., 2018; Masanja et al., 2015; Shekalaghe et al., 2013; Sumari et al., 2016) have demonstrated to deliver high-quality studies outcomes through standard laboratory investigations (Andrew M & Sarah M, 2016). The two mRDTs products; Malaria Pf and Malaria Pf/Pan Antigen Rapid tests manufactured by Zhejiang Orient Gene Biotech Co., LTD detects malaria Plasmodium species antigens. The tests detect malaria antigen in blood specimens from patients presenting malaria symptoms, thereby verifying the presence of P. falciparum and P. vivax, P. malariae and P. ovale malaria infection in humans and Pf/Pan distinguishes Pf infection from non-Pf infection. As with other malaria tests, the result must be interpreted in conjunction with the patient's clinical history. There are several approved mRDTs in the market with sub-optimal performance, since the sensitivity of malaria rapid tests have been reported to vary depending on transmission intensity, whereas in areas with low malaria transmission, RDTs sensitivity have shown to decrease (Taylor et al, 2019; Watson et al, 2019; Hofmann NE et al, 2018). Hence additional mRDTs with improved performance qualifications are warranted. Sensitive mRDTs with the ability to detect low parasitemia of less than 100p/µl and differentiate correctly the P.f

from other species are important in the fight against malaria. Best mRDTs performance will improve rapid case detection, and effective treatment, and prevent long-term complications related to severe forms of malaria and the development of resistant strains, which might be due to incorrect diagnosis and irrational use of drugs. This protocol is intended to perform a field validation of Malaria Pf and Malaria Pf/Pan Antigen Rapid tests manufactured by Zhejiang Orient Gene Biotech Co., LTD

1.2. Study Details

Clinical Trial Registration number	TZ21CT0015
Title of the study	Malaria Pf and Malaria Pf/Pan Antigen Rapid test WHO Prequalification Clinical Study
Protocol Identification Number/code	B1901-05-01
Ethical Clearance Number/ Date of Approval	NIMR/HQ/R.8a/Vol.IX/3644 dated 08/04/2021
TMDA Approval Date	28/6/2021
Name of Investigational Product or Intervention	Malaria Pf and Malaria Pf/Pan Antigen Rapid test
Dosage Form(s) and Strength(s) (where applicable)	N/A
Route(s) of Administration (where applicable)	N/A
Name (s) of Comparator Product (where applicable)	Blood slide microscopy and quantitative Polymerase Chain Reaction (qPCR) will be used to validate the performance of the two test kits however, the qPCR will be used as the reference method.
Name and address(es) of the Sponsor	Zhejiang Orient Gene Biotech Co., LTD LTD 3787#, East Yangguang Avenue, Dipu Street, Anji 313300, Huzhou, Zhejiang, China
Name and address(es) of the Principal Investigator (PI)	Grace Mwangoka Address: Ifakara Health Institute, Bagamoyo Branch Address: P.O.BOX 74 Bagamoyo Contact details: 0766423067
Name and address(es) of Study Site(s)	Name of site: Ifakara Health Institute, Bagamoyo, Kibiti

	Physical address, Box 74 Bagamoyo,
Name and address of the manufacturer of Investigational medical product (IMP) if applicable	Zhejiang Orient Gene Biotech Co., LTD LTD 3787#, East Yangguang Avenue, Dipu Street, Anji 313300, Huzhou, Zhejiang, China.
Name and address of the manufacturer of the comparator product (if applicable	N/A
Phase of Trial	Phase III
Duration of the study	8 Months
Primary purpose of the study (*Screening, Diagnosis, Prevention, Treatment*)	Diagnosis
Condition or diseases under study	Malaria
Number of participants intended to be enrolled in the study	A minimum of 1500 subjects will be enrolled in this study

1.3. Assessment procedure

The application for authorization for a clinical trial of Malaria Pf and Malaria Pf/Pan Antigen Rapid test WHO Prequalification Clinical Study was submitted on 08/04/2021. The assessment was completed in two (2) rounds of evaluation. The trial was approved on 28/6/2021

2. TRIAL INFORMATION

2.1. Study Objectives

Primary Objective

The primary objective of the study is to demonstrate the clinical sensitivity and specificity of the Malaria P.f and P.f/Pan Rapid Test Cassette by trained and untrained operators.

Secondary Objective

The secondary objectives of the study are;

a) To determine the usability of the Malaria P.f and P.f/Pan Rapid Test Cassette in different testing environments (humidity and temperature).
b) To determine the usability of the Malaria P.f and P.f/Pan Rapid Test Cassette in different testing site conditions (indoor/outdoor, lighting, etc.).

2.2. Outcome measures

2.2.1. Primary Outcome

The sensitivity and specificity values were calculated from true and false positives outcomes of the test kits against the standard method which is qPCR and thick blood smears

2.2.2. Secondary Outcome measures

a. Proportions of true positive samples detected by the Malaria P.f and P.f/Pan Rapid Test Cassette in different testing environments (humidity and temperature) using qPCR as a reference method.
b. Proportions of true positive samples detected by the Malaria P.f and P.f/Pan Rapid Test Cassette in different testing site conditions (indoor/outdoor, lighting, etc) using qPCR as a reference method.

2.3. Investigational plan

This is a cross-sectional study design conducted to demonstrate the clinical sensitivity and specificity of the Malaria P.f and P.f/Pan Rapid Test Cassette by trained and untrained operators.

The study will be implemented by Ifakara Health Institute. The study will be conducted in selected areas with different malaria transmission intensities (low and high) to generate robust results for generalization in malaria-endemic settings. Based on the transmission intensity, we will select healthcare facilities at Bagamoyo district and Rufiji and Kibiti districts in the Pwani region and will be carried out for 8 months. All these healthcare facilities will participate in patient enrollment and kit testing in collaboration with IHI-Bagamoyo branch laboratory. Blood samples will be collected from all patients meeting enrollment criteria with suspected malarial infection seeking medical care at sentinel dispensaries and health centers. mRDT testing will be done in the field and main laboratory, while microscopy and PCR will be done at the IHI-Bagamoyo laboratory by qualified technicians.

2.4. Type and number of the study participants

The study plans to enroll about 1500 participants. This includes a total of at least 500 P. falciparum-positive samples and 1000 negative samples from two clinical sites in Africa. Tanzania will enroll 1000 negative samples, 450 P. falciparum positive that includes malaria Pf/pan test kit (200 positives and 500 negatives) and malaria Pf test kit (250 positives and 500 negatives) in a randomized, blind fashion.

2.5. Selection of study population

Inclusion criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply to meet all: -

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

Exclusion criteria

A subject is not eligible for inclusion in this study if any of these criteria meet: -

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

2.6. Drug formulation or device description in case of devices

Product Description for the Malaria P.f. Ag Rapid Test

The Malaria P.f. Ag Rapid Test Cassette (Whole Blood) contains a membrane strip, which is pre-coated with mouse monoclonal antibodies specific to HRP-II of P. falciparum on the test line. The conjugate pad is dispensed with monoclonal antibodies conjugated to colloidal gold, which are specific to P. falciparum histidine-rich protein-2 (Pf HRP-II). During the assay, an adequate volume of the blood specimen is dispensed into the sample well (S) of the test cassette, and a lysis buffer is added to the buffer well (B). The buffer contains a detergent that lyses the red blood cells and releases various antigens, which migrate by capillary action across the strip held in the cassette. pHRP-II is present in the specimen and will bind to the pHRP-II-gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-pHRP-III antibodies, forming a burgundy-coloured band, indicating a positive test result. The absence of a test band suggests a negative result.

Product Description for Malaria P.f./Pan Ag Rapid Test

The Malaria P.f/Pan Ag Rapid Test Cassette contains a membrane pre-coated with mouse monoclonal antibodies specific to HRP-II of P. falciparum at the Pf test line region and with mouse monoclonal antibodies specific to lactate dehydrogenase of Plasmodium species (P. falciparum, P. vivax, P.malariae and P.ovale) at the Pan test line region. The conjugate pad contains a colloidal gold mixture of mouse monoclonal antibodies specific to Pf. HRP-II and mouse monoclonal antibodies specific to pan pLDH. After an adequate

volume of the blood specimen is dispensed into the sample well (S) of the test cassette, the lysis buffer is added to the buffer well (B). The buffer contains a detergent that lyses the red blood cells and releases various antigens, which migrate by capillary action across the strip. Pan-LDH, if present in the specimen, will bind to the Pan-LDH gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-Pan-LDH antibody, forming a burgundy-coloured Pan band, indicating a Pan-positive test result. Alternatively, pHRP-II, if present in the specimen, will bind to the pHRP-II gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-pHRP-II antibody, forming a burgundy-colored Pf band, indicating a Pf-positive test result. The absence of any test (T) bands indicates a negative result. The tests contain an internal control line (C band). After an adequate volume of specimen and buffer is added, a burgundy-colored C-band should appear due to the formation of an immunocomplex of goat anti-mouse IgG/mouse IgG (anti-Pan-LDH and anti-pHRP-II) gold conjugates. If the C-band does not appear, the assay is invalid regardless of colour development at the Pan or Pf test bands and the specimen should be retested with another device.

2.7. Treatments

Treatments administered in case of biologicals or medicines.

2.7.1. The name(s) of all the product(s): N/A
2.7.2. Dose(s): N/A
2.7.3. The dosing schedule(s): N/A
2.7.4. The route/mode(s) of administration: N/A
2.7.5. The treatment period(s): N/A
2.7.6. Follow-up period(s) for participants for each investigational product treatment/trial treatment group/arm of the trial: N/A
2.7.7. Concomitant Medication(s)/treatment(s) permitted (including rescue medication) and not permitted before and/or during the trial: N/A
2.7.8. Procedures for monitoring participant's compliance: N/A
2.7.9. Wash-out period (Description for pre-, during- and post-trial, as applicable) N/A

2.8. Pre-study screening and baseline evaluation

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

2.9. Efficacy and safety measurements to be assessed

Efficacy measurements
Positive likelihood ratio = sensitivity/(1-specificity)
Negative likelihood ratio = (1-sensitivity)/specificity
Safety measurements
Not applicable

3. ETHICAL CONSIDERATIONS

3.1. Ethical Clearance

The study has approved by the National Health Research Ethics Committee on 08 April 2021 with ethical clearance. No NIMR/HQ/R.8a/Vol.IX/3644 dated 08/04/2021

3.2. Insurance

N/A

3.3. Informed Consent

Informed consents version No.1 was approved by the ethics committee on 08/04/2021

3.4. Patient Information Leaflet

Patient information leaflet version No. 1 was provided as approved by the ethics committee on 08/04/2021

3.5. Payment

The study participants will receive a token amounting to TZS 5000 as reimbursement for their time, transport and inconveniences at the study visit

4. WHAT ARE THE BENEFITS OF BEING IN THE STUDY?

The study participants will not have immediate benefits directly from the study. However, when the study results are known and the rapid tests 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.

5. ARE THERE ANY POTENTIAL BENEFITS TO OTHERS THAT MIGHT RESULT FROM THE STUDY?

In future, other people might benefit from this study because the information may help us learn more about diagnosing and treating malaria infection.

6. WHAT ARE THE RISKS OF THE STUDY?

The study doesn't pose any risks when you participate in the study, however, you may feel a small amount of discomfort or have a small amount of bruising on your finger and arm where the blood will be taken.

Details on treatment and/or management of participants and their disease condition(s) after completion of the trial (Post-trial medicine access) if provided

N/A

7. PRE-CLINICAL STUDIES (IF APPLICABLE)

N/A

8. HUMAN EXPERIENCE (CLINICAL STUDIES) IF APPLICABLE

N/A

9. PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION OF THE INVESTIGATIONAL MEDICINAL PRODUCT OF DRUG SUBSTANCE (VACCINES AND DRUGS ONLY)

9.1. Drug Substance(s)

9.1.1. Description

N/A

9.1.2. Name(s)

N/A

9.1.3. Structural Formula and Molecular Formula

N/A

9.1.4. Physical-Chemical Properties

N/A

9.1.5. Drug Substance Stability

N/A

9.2. Drug Product

N/A

9.2.1. Drug Product Formulation

N/A

9.2.2. Placebo Formulation (In case applicable)

N/A

9.2.3. Drug Product Stability

N/A

9.2.4. Drug Product Storage

N/A

10. BENEFIT-RISK ASSESSMENT AND CONCLUSION

N/A

11. POST-APPROVAL UPDATES

N/A

11.1. Amendment applications

Reference number	Date submitted	Change requested	Recommendation	Granting date

PART 5: CHANGE HISTORY

Version number	**Date**	**Description of update**	**Section(s) Modified**	**Approval date**