

1. Protocol title

Title: Evaluation of the performance of a highly-sensitive rapid diagnostic test (hsRDT) *versus* conventional RDT (cRDT), compared with polymerase chain reaction (PCR) as the gold standard, in reactive case detection of malaria infections in Rakhine State, Myanmar

Protocol Number: TBD

Protocol version Number: Draft version 0.4

Day Month Year: 3 July 2017

Abbreviations

ACT	Artemisinin-based Combination Therapy
CNP	Copy Number Polymorphism
DBS	Dried Blood Spot
DMR	Department of Malaria Research
DNA	Deoxyribonucleic acid
ERC	Ethics Review Committee
GCP	Good Clinical Practice
GMS	Greater Mekong Sub-region
GWAS	Genome-Wide Association Study
ICH	International Conference on Harmonization
ID	Identification
N	Number (typically refers to subjects)
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PMI	U.S. President's Malaria Initiative
SOP	Standard Operating Procedure
UMB	University of Maryland, Baltimore
URC	University Research Co.
USA	United States of America
USAID	United States Agency for International Development
VBDC	Vector Borne Disease Control
WHO	World Health Organization
WWARN	Worldwide Antimalarial Resistance Network

Protocol Summary

- Title:** Evaluation of the performance of a highly-sensitive rapid diagnostic test (hsRDT) *versus* conventional RDT (cRDT), compared with polymerase chain reaction (PCR) as the gold standard, in reactive case detection of malaria infections in Rakhine State, Myanmar
- Population:** Adults and children at least 6 months of age in a specified study site
- Number of Sites:** One
- Study Duration:** One year
- Subject Duration:** One encounter lasting no more than 30 min

Study objectives:

General objective: To evaluate the performance of the highly-sensitive rapid diagnostic test (hsRDT), developed by SD Bioline, *versus* conventional RDT (cRDT), compared with PCR as the gold standard, in reactive case detection to identify additional malaria infections in Rakhine State, Myanmar

Specific objectives

1. To evaluate the prevalence of malaria identified by the new hsRDT in comparison with that by cRDT and PCR
Outcomes: test positivity rate by cRDT, hsRDT and PCR, respectively
2. To assess the diagnostic performance characteristics of hsRDT *versus* cRDT, using PCR as gold standard, in the detection of *P.falciparum* infections
Outcomes: Sensitivity, specificity, positive and negative predictive value of hsRDT and cRDT
3. To evaluate correlation of detection capability between cRDT and hsRDT
Outcomes: Correlation of test positivity rate by the three different study tests
4. To identify risk factors associated with malaria infection, including but not limited to, socio-demographic factors and travel history related with malaria index cases
Outcomes: Relative risk of malaria in association with different risk factors identified

2. Investigators, institutional affiliations, and role

Principal Investigator:

San Kyawt Khine
Assistant Director
Vector Borne Disease Control (VBDC) Rakhine State
Department of Public Health
Myanmar Ministry of Health and Sports (MoHS)

Co-investigators:

Dr. Aung Thi	Program Manager, National Malaria Control Program (NMCP), Myanmar
Dr. Aye Nyein	Team Leader, Vector Borne Disease Control (VBDC) Team, Rakhine State
Dr. Nay Yi Yi Linn	Assistant Director, NMCP
Dr. Zay Min Tun	Medical Officer, NMCP
Dr. Saw Lwin	Deputy Chief of Party & Technical Advisor, Defeat Malaria, University Research Co. (URC)
Dr. Feliciano Monti	Senior Malaria Advisor, U.S. President's Malaria Initiative (PMI), U.S. Agency for International Development (USAID)
Dr. Jimmie Hwang	Medical Epidemiologist, U.S. Center for Disease Control & Prevention (CDC)
Dr. Nelli Westercamp	Epidemiologist, U.S. Center for Disease Control & Prevention (CDC)
Dr. Khin Than Win	Senior Technical Director, Defeat Malaria, URC
Dr. Kyaw Myint Tun	Monitoring & Evaluation (M&E) Technical Advisor, Defeat Malaria, URC
Dr. Kay Thwe Han	Deputy Director, Parasitology Division, Department of Medical Research, Myanmar MoHS
Dr. Myaing M. Nyunt	Director, Institute for Global Health (IGH) Myanmar, University of Maryland Baltimore (UMB)
Dr. Janie Zuber	Infectious Disease Fellow, IGH, UMB
Dr. Christopher Plowe	Director of IGH & Professor of Molecular Epidemiology & Infectious Diseases, UMB
Dr. Yuanyuan Liang	Professor of Biostatistics, IGH, UMB
Matt Adams	Lab supervisor, UMB

Sponsor: U.S. President's Malaria Initiative

For questions regarding this protocol, contact:

Individuals: Sponsor representative

Dr. Feliciano Monti
U.S. President's Malaria Initiative
U.S. Embassy, Yangon
Tel. +95-(0)9-5419651
Email: fmonti@usaid.gov

Principal Investigators:

Dr. San Kyawt Khine
Assistant Director
Vector Borne Disease Control (VBDC) Program, Rakhine State
General Hospital Compound, Sittwe, Rakhine State
Tel. +95-(0)9-450542076
Email: skkhine.khine75@gmail.com

Senior Technical Advisor

Dr. Saw Lwin
Deputy Chief of Party
University Research Co. (URC), Defeat Malaria
Room 602/701, Shwe Than Lwin Condominium, New University Avenue
Road, Bahan Township, Yangon]
Tel. +95-(0)9-5014887
Email: slwin@URC-CHS.COM

Participating Institutions:

Vector Borne Disease Control Program

Diseases Control Office,
Zabu Thiri Township
Nay Pyi Taw, Myanmar]
Tel. 067-421204
Fax 067-421204

Department of Medical Research

Ministry of Health and Sports
5, Ziwaka Road, Dagon P.O.
Yangon 11191, Myanmar
Tel. +95-1-380129
Fax. +95-1-251514

University Research Co. Myanmar

Room 602/701, Shwe Than Lwin
Condominium,
New University Avenue Road,
Bahan Township, Yangon
Tel. +95-1-543353
Fax: 01-1-220658

**U.S. Centers for Disease Control and
Prevention**

1600 Clifton Rd, Atlanta, GA, 30333, USA
Tel. +1-770-488-7100
Fax +1-770-488-4206

Institute for Global Health

University of Maryland School of Medicine
485 West Baltimore Street, HSFII-480
Baltimore, Maryland 21201 USA
Tel. +1-410-706-2491
Fax. +1-410-706-1204

Co-investigators and their role

Name	Institution	Role	Brief description
Dr. San Kyawt Khaing	VBDC-Rakhine	Principle Investigator	Responsible for the overall technical, regulatory, financial and ethical adequacy of the study; provide necessary administrative and technical support for study team; Respond timely and adequately for the needs from study team; Preparation of study protocol and related documents; Ensure accuracy and completion of all study related documents, and study samples and data timely.
Dr. Saw Lwin	URC Defeat Malaria	Co-investigator	
Dr. Aung Thi	NMCP	Co-investigator	Overall administrative and technical leadership; Guidance for study conduct and decision on interpretation and implication of study findings
Dr. Aye Nyein	VBDC-Rakhine	Team leader	Responsible for leading and managing daily activities of study team; Guide and coordinate issues related to local circumstances and overall organization of implementation of the study and responsible for all communications with the authorities concerning the study.
Dr. Nay Yi Yi Linn	NMCP	Assistant Director	Assist Program Manager of NMCP in critically reviewing the paper
Dr. Zay Min Tun	NMCP	Medical Officer	Assist Program Manager of NMCP
Dr. Feliciano Monti	PMI	Senior Malaria Advisor	Provide guidance for administrative and regulatory adequacy of study conduct; Preparation of study protocol and related documents
Dr. Jimee Hwang	CDC	Medical Epidemiologist	Provide guidance for scientific and technical adequacy of study design, preparation and conduct, and for study data analysis and interpretation
Dr. Nelli Westercamp	CDC	Epidemiologist	

Dr. Khin Than Win	URC Defeat Malaria	Co-investigator	Oversee and ensure technical adequacy of the study team and responsible for overall accomplishment of the study by advocacy to responsible persons at all level, seeking approval from Department of Public Health, engage the research team with community, arrangement of specimen for proper storage and transport.
Dr. Kyaw Myint Tun	URC Defeat Malaria	Co-investigator	Support successful submission of protocol to Ethics Review Committee, recruit and organize survey team, plan and facilitate necessary trainings for implementation of operational research, site selection for study villages, monitoring and supervision of survey activities, ensure data quality for analysis, and data validation.
Dr. Kay Thwe Han	DMR	Co-investigator	Oversees and lead all aspects of lab work including sample management; Co-train with UMB the study teams; Provide research and lab support for study team
Dr. Myaing Myaing Nyunt	IGH-UMB	Co-investigator	Responsible for all communication and coordination between UMB, URC and DMR; Design and prepare study protocol and related documents; Assist NMCP and URC for admin and ethical approval; Ensure timely and adequate preparation and completion of laboratory work; data analysis, interpretation and manuscript preparation; Assist NMCP and URC for study related matter, data dissemination.
Dr. Chris Plowe	IGH-UMB	Supervising investigator/ Molecular Epidemiologist	Provide overall administrative and technical expert opinion; support for study epidemiologic design, molecular aspect of study conduct and analyses; statistical consideration, lab analysis, data interpretation and dissemination
Matt Adams	IGH-UMB	Lab supervisor	Responsible for readiness of all field and lab supplies and equipment & Quality control & assurance; Train lab team; Oversees lab analysis; problem solves and act according to results and needs; Train, manage and supervise lab data analysis; Ensure timely completion of lab work and data dissemination

Dr. Janie Zuber	IGH-UMB	Infectious Disease Research fellow	Assist protocol development, study planning and conduct, data organization, analysis and interpretation; Manuscript preparation; Work under Nyunt's supervision
Dr. Yuanyuan Liang	IGH-UMB	Statistician	Responsible for sampling and statistical procedures, analysis, interpretation of results and formulation of recommendations. Advice on study design, data analysis and interpretation; manuscript preparation

3. Background information and scientific rationale

3.1. Background and introduction

Myanmar, along with several other countries in the Greater Mekong Sub-region (GMS), is committed to eliminate malaria by 2030. In the National Plan for Malaria Elimination in Myanmar (2016–2030), the NMCP recommended that standard operating procedures (SOPs) for malaria active case detection (ACD) should be developed, and that the role of rapid diagnostic tests (RDTs), microscopy, and PCR-based techniques in ACD at different levels of the transmission-reduction and elimination phases be evaluated.

Recent epidemiological data indicates that 30-50% of all malaria infections exist in a form of asymptomatic infection (also known as “chronic malaria”) where the parasite density is generally below the limit of detection of all point-of-care diagnostic tests [1]. The requirement for more sensitive diagnostic tests for malaria is predicated on the idea that malaria elimination may require eradicating all parasites from all infected persons, not just those with clinical symptoms [2]. “Asymptomatic”—or more accurately, *subclinical* malaria—has long been recognized in semi-immune Africans [3], but not in Southeast Asia where malaria transmission is generally lower. Many believed that subclinical infections are unlikely in low transmission settings, partly due to a long held dogma that partial immunity does not develop to allow for such low grade infections, and partly to the lack of detection methods adequately sensitive to identify very low parasite density typically found in the GMS [4-6].

New “ultrasensitive” PCR-based tests, with a lower limit of detection 16–22 parasites/mL [4, 7-9], have recently found unexpectedly high rates of subclinical malaria in Southeast Asia. These infections are missed by standard diagnostics such as microscopy or conventional RDT which have a lower limit of detection about 100 parasites/uL. Though the PCR is adequately sensitive to detect these low-density infections, its utility is quite severely limited by its complexity, requirement for advanced skills and infrastructure, cost, and by the fact that it is not a point-of-care test. Today’s malaria elimination toolkit essentially consists of prompt diagnosis and treatment using rapid diagnostic tests (RDTs) and artemisinin-based combination treatments (ACTs), vector control methods, and surveillance using RDTs and/or microscopy. The available diagnostic tests are inadequate to detect these low-density malaria infections, and new tools are urgently needed.

Three studies, a survey, and a systematic review assessing the role, appropriateness and benefits of the ACD strategy, both proactive and reactive, in low transmission settings have been recently conducted in Cambodia, Indonesia, Thailand, India and other Asian-Pacific countries [10-12]. The data clearly indicated that the ACD strategy needs to be optimized based on the local context, and on evidence for the adoption of improved methods. The need for better, cheaper and more accurate diagnostic tools, with an increased capacity to detect low density malaria infections, was highlighted in recent literature.

To fill this critical gap, a new hsRDT that has a potential to be at least 10- to 20-fold more sensitive for detecting low density infections than standard cRDT was developed (Standard Diagnostics Bioline Inc. Gyeonggi-do, Republic of Korea), and is now under field evaluation in several malaria endemic countries. This test is similar in design to cRDT, using a handheld point-of-care laminar flow test to detect low levels of the *P. falciparum* antigen Histidine Rich Protein 2 (HRP2), which is also one of the detection targets of cRDT. Initial prototype testing of the new hsRDT designed to detect the *P. falciparum*-specific antigen HRP2 in a format

similar to the current cRDT resulted in a limit of detection (LOD) of *HRP2* of about 80 pg/mL compared to cRDTs with a LOD of 800–1000 pg/mL (personal communication, S. Allauzen, Diagnostics & Global Health, Bill & Melinda Gates Foundation).

We propose to evaluate the role of this new hsRDT in the setting of reactive case detection (RCD) in specified townships where malaria prevalence has been steadily declining. Our primary goal is to assess whether or not this new hsRDT performs significantly better than cRDT in identifying subclinical submicroscopic infection, and to subsequently evaluate if hsRDT can be used for effective ACD in Myanmar. The proposed study may shed light on the development of national standard operating procedures (SOPs) for ACD as recommended by the NMCP. The development of these SOPs are ongoing, and our study may provide timely and relevant contribution for their development, particularly assessing the detection accuracy and utility of the new hsRDTs when used as a screening tool in the RCD strategy. Additional useful information can be obtained on the applicability, cost, and operational challenges faced during the field implementation.

The proposed study also addresses PMI elimination or priority to “Evaluate the utility and scalability of more sensitive diagnostic tools (e.g. highly-sensitive RDTs, PCR, etc.) in an elimination context, including their utility for reactive or proactive case detection.” It will also contribute to current PMI research activities comparing RCD with RDTs to focal Mass Drug Administration (MDA) planned for Madagascar and Ethiopia. The Madagascar study will also include the hsRDT in their RCD arm. If the test of this hsRDT in detecting additional malaria infections around index cases shows good field performance, further trials can be planned in Myanmar to evaluate its role in malaria elimination.

1. Rationale for the study

Preliminary data in the development of a new hsRDT indicated that this hsRDT has a significantly higher sensitivity than cRDT and will perform better in detecting low density malaria infections (Personal communication; Jonathan Cox, Bill and Melinda Gates Foundation), and we anticipate that it may play a critical role in reactive case detection strategy. The current tools such as microscopy or cRDT are insufficient in identifying these infections and missing them may pose a great drawback for the progress of preparation for malaria elimination. In addition to its high sensitivity, hsRDT is a point of care test, therefore it is convenient for large scale testing and sustainable in resource-limited settings. This tool is urgently needed in the current surveillance activities in Myanmar during the time of preparation for the pre-elimination and subsequent elimination phase. Findings from the proposed study will also contribute useful data in the development of national standard operating procedures for ACD with potential value of the new hsRDTs as a screening tool in the RCD strategy.

3.3. Potential Risks and Benefits

3.3.1. Potential risks

Effects of finger sticks. Very small risks of bruising, bleeding, infection and fainting can accompany these procedures. Study-related blood collection for malaria diagnostic testing (blood smear on microscope slides or rapid diagnostic test) and dried blood spot sample can be accomplished by using finger-stick.

Potential risks to study personnel. The main risks to study personnel are from accidental exposure to blood and body fluid borne infections. SOPs for staff safety are

used in clinical and laboratory areas, including sharps management, hazardous waste management, etc. Universal precautions are used for handling all body fluids.

Because the described analyses pose minimal appreciable additional risks to the trial participants but present potential benefits to their communities and others living in malaria endemic areas, the risk-to-benefit ratio is very low.

3.3.2. Known potential benefits

Participants may not receive any direct benefit from their participation in the study. However, if symptoms of other illnesses are noted, participants will be referred for evaluation and treatment at the appropriate local clinic.

If malaria parasites were identified by cRDT or hsRDT, participants will receive appropriate anti-malaria treatment promptly and potential transmission will be interrupted, benefiting the community. The benefits to the community could include improved tools for surveillance and diagnosis of malaria. Results from this study may help malaria control programs improve understanding the degree and distribution of “silent” reservoir that cannot be readily detected using current diagnostic tests.

4. Study objectives

General objective: To evaluate the performance of the highly-sensitive rapid diagnostic test (hsRDT), developed by SD Bioline, versus conventional RDT (cRDT), compared with PCR as the gold standard, in reactive case detection to identify additional malaria infections in Rakhine State, Myanmar

Specific objectives:

1. To evaluate the prevalence of malaria infections identified by the new hsRDT in comparison with that detected by cRDT and PCR
Outcomes: malaria test positivity rate by cRDT, hsRDT and PCR, respectively
2. To assess the diagnostic performance characteristics of hsRDT *versus* cRDT, using PCR as gold standard, in the detection of *P.falciparum* infections
Outcomes: Sensitivity, specificity, positive and negative predictive values of hsRDT and cRDT
3. To evaluate correlation of detection capability between cRDT and hsRDT
Outcomes: Correlation of test positivity rate by the three different study tests
4. To identify risk factors associated with malaria infection, including but not limited to, socio-demographic factors and travel history related with malaria index cases
Outcome: Relative risk of malaria in association with different risk factors identified

5. Methodology

5.1. Study Design & Overview

This is a prospective community-based single-center reactive case detection (RCD) study to assess the performance of hsRDT versus cRDT in identifying individuals with malaria infection (“Secondary case”) in a population living and/or working in a close physical proximity to an “index case.”

“Index case” is defined as a malaria infection identified by passive surveillance by local health services (rural health center, sub-centers) or village malaria workers (VMWs), by cRDT or microscopy.

“Secondary case” is defined as any malaria infection identified by any of the study diagnostic test: cRDT, hsRDT or PCR, regardless of the presence of acute clinical illness. The secondary cases will be identified actively and systematically around the residence, or work place if relevant, of the index case.

The study will be initiated after obtaining administrative (Myanmar MoHS, NMCP, PMI) and ethical approval (Myanmar DMR ERC, CDC IRB and UMB IRB) from all relevant institutions. Consent, and assent and adult permission when appropriate, will be sought for all index cases and secondary cases. Only those who are eligible and provide informed consent will be enrolled in the study.

All index cases parasitologically confirmed by cRDT or microscopy will be appropriately and promptly notified to the study team, using mobile phone technology where possible, to ensure timely enrollment. The home of the index case, or work place when relevant, will be visited within three days of the diagnosis, and s/he will be interviewed with a standardized case investigation form. During the visit, all members of the index case household and all members of the nearest 10 households, aged 5 years or above, will be invited to participate in the study. All participants in the study will be interviewed with a structured questionnaire, tested with cRDT and hsRDT, and a dried blood spot (DBS) will be collected for subsequent PCR analysis. All cases parasitologically confirmed by cRDT or hsRDT will be appropriately treated, following the national treatment guideline. DBS samples will be collected, processed and stored until analysis following the World Wide Antimalarial Resistance (WWARN) Molecular Testing for Malaria Standard Operating Procedures DBS Sample Collection and Transportation (<http://www.wwarn.org/learning/procedures/dbs-sample-collection-and-transportation>).

The prevalence of secondary malaria cases (number positive /number tested), by cRDT and hsRDT will be compared, using PCR as gold standard. Using simple analysis of performance characteristics, the sensitivity and specificity of hsRDT will be estimated in the study population. Potential correlation between the detection rate by cRDT, hsRDT and PCR will be assessed.

5.2. Study Period

The study will be conducted from June, 2017 to May, 2018, after obtaining administrative and ethical approval from appropriate authorities.

5.3. Study Area

The proposed study will be conducted in the village tract of Sakhanmaw, in Ann township in South Rakhine State. This village tract encompasses 63 villages located in a hilly forested area

with small rivers and valleys, with a total population of about 23,000 residents. Sakhamaw village tract is covered by one rural health center (RHC) and seven sub-Centers. In addition to basic health services, malaria volunteers conduct malaria case detection and management in 52 villages. The volunteers are trained by National Malaria Control Program, University of Research Cooperation and Myanmar Medical Association.

Of the 63 villages, the research study will be carried out in about 35 villages with low malaria transmission with an annual parasite incidence (API) less than 5 per 1,000 population at risk. Based on the epidemiological data collected during 2016, the test positivity rate in the study villages ranged from 0% to 13%, and more than 80% of the malaria infections were caused by *P. falciparum*. The primary malaria vectors are *Anopheles minimus* and *An. dirus*.

5.4. Study population

All adult and children at least 5 years old in the study villages under Sakhamaw RHC will be recruited and enrolled in the study.

Inclusion criteria

- Age at least 5 years old
- Resident of the villages, or temporary visitors, or co-workers or co-travelers of the index case
- Willingness to participate in the study evident by informed consent

Exclusion criteria

- Presence of severe clinical illness including severe malaria
- Non-resident index cases
- Refusal to participate in the study

Initial meetings will be organized with all partners involved in the research (NMCP, VBDC Rakhine, DMR, Defeat Malaria partners, local authorities and community leaders) to clarify the scope of the study, and prompt their active collaboration.

Additionally, specific information and sensitization activities will be organized for the communities involved in the study to explain the nature of the research, address possible concerns, and encourage their motivated participation.

5.5. Recruitment Procedures, Identification index cases and selection of secondary cases

Suspected malaria cases attending local health services (rural health center and sub-centers) or village malaria workers (VMW) will be tested with a cRDT as in routine practice. All cases parasitologically confirmed by cRDT will be treated according to national guidelines, and asked for informed consent to participate in the study. If the patient gives informed consent, the VMW or basic health staffs will promptly notify the study team, using mobile phone. Additionally, finger prick blood will be collected from the index case to test with hsRDT, and on dry blood spot (DBS) for later PCR analyses.

The study team will conduct the investigation visit at the residence of the index case within 3 days, and the index cases will be interviewed using a standard case investigation form (Annex 1). All members of the index case's household will be invited to participate in the study and

interviewed using the participants' questionnaires (Annex 2) to assess a history of malaria in previous year, travel information, and utilization of malaria prevention measures. The nearest ten households around the index case's household will be identified by using household listing. All members, including temporary visitors, of these ten households will be invited to participate in the study and interviewed using the participants' questionnaires (Annex 2), tested with cRDT and hsRDT, and a DBS will be collected for subsequent analysis. If the index case reports traveling to or working in the forest or other risk areas, co-travelers or co-workers will be also recruited in the study, when feasible.

Study participants who tested positive on cRDT or hsRDT will be treated according to national guidelines, in compliance with WHO recommendations and NMCP policies, which call for treatment of any RDT-positive infection. Although the diagnostic performance of hsRDT is under investigation, there is enough evidence that cRDT fails to detect low density infections, therefore treatment of hsRDT-positive malaria is conservative and ethically justifiable.

PCR results will not be available immediately, and there are no recommendations for retrospective malaria treatment based on ultrasensitive testing. Those who are ill but found to be negative on RDTs will be provided with information to seek further care and referral to an appropriate medical professional will be made. Study team will be equipped with basic medical supplies and provide as much care as possible.

5.6. Seeking Informed Consent/Assent from potential participants

Prior to the study data and sample collection, preliminary discussion meetings will be organized with all partners involved in the research (NMCP, VBDC Rakhine, DMR, Defeat Malaria partners, local authorities and community leaders) to explain the nature and scope of the study, and prompt their active collaboration. Community outreach activities will be conducted to ensure the community response and study participation. Study time line will be disseminated to the targeted communities in a locally appropriate manner.

The principles of research ethics in the current edition of the Declaration of Helsinki will be applied, and protocol-specified procedures will be initiated only after obtaining informed consent. The written consent documents will embody the elements of informed consent as described in the current edition of the Declaration of Helsinki, will adhere to the ICH Harmonized Tripartite Guideline for Good Clinical Practice and 45CFR46 and 21CFR50, and will also comply with applicable Myanmar regulations. The oral consent process will be consistent with 45CFR46. §46.117, 21CFR50.27 and ICH E6 (R1) Section 4.8.

Information about the study will be given to prospective participants, in a simple lay language both in Myanmar and Rakhine, in both oral and written forms whenever possible. Independent witnesses will be used to attest that illiterate potential participants have understood the contents of the informed consent document.

The UMB investigators and staff will closely work with the NMCP and other relevant local research staff at the study sites, providing training, and re-training if necessary, in Protection of human subjects, Good Clinical Practice, and responsible conducts of research to assure study information and informed consent procedures meet requirements of the community, sponsor and all relevant ethic review oversight committees. UMB investigators have published detailed descriptions of the processes that have been used to obtain community "permission to enter" and individual informed consent [42]. We consider informed consent to be a dynamic, ongoing

process, with continuous availability of investigators to answer any questions that arise in the course of the study and to ensure that participants and their parents/guardians understand study procedures. Even though subject participation will be brief for this study, these same principles will be followed.

Since the vast majority of study participants' parents/guardians do not use telephones, fax or mail, contact information is provided in terms of local physicians who can be visited directly and who can themselves reach the investigators directly or by telephone or fax.

In Myanmar, individuals who are at least 18 years old are considered adults and will provide consent for themselves; children 6 months to ≤ 7 years of age are too young to provide assent; however, written consent will be provided by their guardian; children 8 to ≤ 12 years of age will provide verbal assent, in addition to written consent provided by their guardian; children 13 to ≤ 17 years of age will provide written assent, in addition to written consent provided by their guardian. In rural Myanmar guardians need not necessarily be the child's parent(s); Any close relative including aunts, uncles, grandparents, adult siblings etc. who accompany the child to the clinic may serve as the guardian for the sake of providing informed consent. This may include neighbors or village elders in the case of orphans.

5.7. Data and Blood Sample Collection

Participant will be given a unique study identification number which, instead of the participant's name or other personal identification, will be used for his/her case record forms, other study related documents and for labelling the study samples. This will minimize a potential loss of confidentiality for the participant.

A trained study personnel will clean with antiseptic, and prick the tip of the participant's finger (or the heel if and when appropriate for children 6–12 months old) using a lancet, and collect 5-7 drops of blood (a quarter of teaspoon). Two to three drops will be used to check for malaria by cRDT and hsRDT, and 3-4 drops will be collected on to filter papers for malaria PCR testing. The filter paper will be placed at the prick site on the finger to absorb the blood. For infants 6 months to one years of age, a heel prick might be more appropriate.

The PCR analysis will be carried out at the DMR in Yangon, with technical assistance and quality management from the University of Maryland. Additional molecular, antigen and antibody-based testing for malaria may be conducted depending availability of funding. The DBS, packed in individual plastic bags with desiccant, will be appropriately stored at Defeat Malaria's field office in Ann Township and sent to DMR in Yangon every 2-3 weeks.

6. Sample Management and Laboratory Evaluation

6.1. Specimen Collection, Preparation, Handling and Shipping

DBS specimens will be collected using standard methods (see <http://www.wwarn.org/learning/procedures/dbs-sample-collection-and-transportation>). DBS samples will be labeled with participant unique ID. No names and other personal identifiers will be recorded. After collection, DBS will be air dried and stored in zip-lock plastic bags with desiccant at each site (see Appendix B: Molecular Testing for Malaria Standard Operating Procedures DBS Sample Collection and Transportation) at +4 °C, -20 °C or room temperature in a cool, dry, dark location.

6.2. PCR assay from dried blood spot

DNA will be extracted dried blood spots using a validated new extraction method developed by our group (Zainabadi et al. “A dried blood spot based ultrasensitive detection method for asymptomatic malaria” Oral presentation at the 57th annual meeting of American Society of Tropical medicine and Hygiene, November 2016). As much of the molecular analyses as possible will be done at the Advanced Molecular Research Center of the Department of Medical Research, under the Myanmar Ministry of Health. Extracted DNA will be subjected to ultra-sensitive qPCR using well-established techniques that currently have a lower limit of detection of 22 parasites/mL (9). PCR will be done in Myanmar with some duplicate samples analyzed at the University of Maryland for quality control purposes.

Future studies of parasite DNA derived from samples may be conducted without restriction. ***No other studies on any topics beyond what is described in this protocol and consent/assent forms or on human DNA will be done unless specific permission for additional studies is obtained from the relevant IRBs.*** Study participants will have the right to withdraw their permission for further use of their samples at any time during and after the study.

Samples will be batched and shipped periodically to the DMR laboratory and/or the laboratories at the University of Maryland in Baltimore or CDC-Atlanta. DBS specimens will be shipped at room temperature and desiccant will be replaced upon receipt. Specimen receipt will be logged using a system designed at UMB using the Freezer works program. The laboratory contact at DMR is Dr. Kay Thwe Han, E-mail: drkaythwehan@yahoo.com. The laboratory contact at UMB is the lab supervisor Matthew Adams E-mail: madams@som.umaryland.edu.

7. Statistical consideration and Data Management

7.1. Power calculation with available sample size

The primary limitation is the size of population that needs to be screened since malaria burden may be low in the study sites, and associated financial costs and logistical difficulties.

The primary endpoint of this proposed study is the prevalence of secondary malaria cases identified by hsRDT in comparison with usPCR prevalence. Based on our current data on prevalence of *P. falciparum* in 2016, and logistical and financial considerations, the recruitment of 50 index cases is feasible in a given study period. This will give us a total of 1980 individuals for testing of secondary cases in ACD, assuming the average household member of 4.5, 11 households for each index case (counting the household of the index case) and a refusal rate of 20% (4.5 persons/HH x 11 HH x 50 index cases x 0.80=1980).

We conservatively estimate that hsRDT will detect 20% of usPCR+ *P. falciparum* infections (i.e., sensitivity of hsRDT=0.2 using usPCR as gold standard). We expect very few recently treated individuals to be enrolled in the study, so that hsRDT should only rarely give a positive result when usPCR does not. Hence we assume that the specificity of hsRDT is 0.995. Table 1 shows estimated statistical power that can be achieved with the proposed sample size of 1980 for comparing the prevalence by hsRDT and the prevalence by usPCR, at different malaria prevalence by usPCR and different sensitivity of hsRDT, using a two-sided McNemar test with a Type I error of 5%. (PASS version 11. NCSS, LLC. Kaysville, Utah, USA)

As shown in Table 1, if the sensitivity of hsRDT is 0.2 and the specificity of hsRDT is 0.995, a sample size of 1980 subjects achieves at least 92% power to detect the difference in prevalence between hsRDT and usPCR when the malaria prevalence is 2% or higher. If the sensitivity of hsRDT is 0.5 (see the last four columns of Table 1), a sample size of 1980 subjects achieves at least 86% power when the malaria prevalence is 3% or higher. If the prevalence of malaria is higher, the performance of hsRDT will be better with a higher sensitivity and positive predictive value.

Table 1. Statistical power expected with proposed sample size of 1980 at different malaria prevalence and different sensitivity of hsRDT (assumed sensitivity of 20% or 50% and specificity 99.5%)

Prevalence by usPCR	Sensitivity of hsRDT	Specificity of hsRDT	Estimated prevalence by hsRDT	Power	Sensitivity of hsRDT	Specificity of hsRDT	Estimated prevalence by hsRDT	Power
0.02	0.2	0.995	0.009	0.92	0.5	0.995	0.015	0.45
0.03	0.2	0.995	0.011	>0.99	0.5	0.995	0.020	0.86
0.06	0.2	0.995	0.017	>0.99	0.5	0.995	0.035	>0.99
0.12	0.2	0.995	0.029	>0.99	0.5	0.995	0.065	>0.99

Table 2 below shows changes in the power of the study, if the specificity of hsRDT is 0.95 (increased false positive by hsRDT), while the sensitivity of hsRDT and baseline true prevalence remain the same.

Table 2. Statistical power expected with proposed sample size of 1980 at different malaria prevalence and different sensitivity of hsRDT (assumed sensitivity of 20% or 50% and specificity 95%)

Prevalence by usPCR	Sensitivity of hsRDT	Specificity of hsRDT	Estimated prevalence by hsRDT	Power	Sensitivity of hsRDT	Specificity of hsRDT	Estimated prevalence by hsRDT	Power
0.02	0.2	0.95	0.009	0.053	0.5	0.95	0.059	>0.99
0.03	0.2	0.95	0.011	0.055	0.5	0.95	0.064	>0.99
0.06	0.2	0.95	0.017	0.059	0.5	0.95	0.077	0.79
0.12	0.2	0.95	0.029	0.068	0.5	0.95	0.104	0.61

7.2. Analytical Plan

All data will be double-entered into Epi-Info and any discrepancies will be reviewed against the paper forms. All analyses will be conducted using Stata/SE (version 14), using data from the participant interviews and the three parasitological tests.

The malaria test positivity rate will be calculated as a proportion of samples tested that are positive using cRDT, hsRDT and PCR, respectively. Corresponding 95% confidence intervals (CIs) will be computed using exact method. A 2x2 table method will be used to calculate the sensitivity, specificity, positive and negative predictive values of hsRDT (and cRDT) and their corresponding 95% CIs using PCR as gold standard. The malaria prevalence by each test (e.g., the rate of malaria test positive) will be compared between each two tests (hsRDT vs PCR; cRDT vs PCR; hsRDT vs cRDT) using McNemar test. All statistical tests will be performed with a two-sided significance level of 0.05.

Descriptive analysis will be conducted to assess socio-demographics characteristics. Univariate analysis will include t-test and non-parametric methods for continuous variables and chi-square test for comparison of categorical variables. Multi-level regression models will be developed to examine risk factors significantly associated with malaria infections, accounting for clustering by index case.

7.3. Data Management

The principal investigator will ensure that the study protocol is strictly adhered to and that all data are collected and recorded correctly on the data collection forms and logs. Any change or correction to a form should be dated and explained and should not obscure the original entry. All forms will be checked for completeness. The paper forms will be kept in a locked file cabinet when not in use.

1. Data Collection Method

- Record review of malaria case registers of village malaria workers and health facilities
- Face to face interview of index case, all members of index case household members and all members of nearby 10 households
- Finger prick blood sample collection for cRDT, hsRDT, DBS collection of all participants including short face to face interview to be recorded on the household screening form

2. Data Collection Tool

- The routine malaria surveillance data from the study villages will be collected from malaria patient register used by volunteers or basic health staffs.
- Index case will be interviewed and recorded by respective VMWs/BHS using case investigation form (Annex 1) and household listing and participant questionnaire form (Annex 2).
- All the index household members and nearby household members will be interviewed and recorded by respective VMWs/BHS using household listing and participant questionnaire form (Annex II).
- Information of results of the conventional RDT and hsRDT screening as well as personal risk factors such as travel history, forest related work, use of malaria prevention tools, etc. will be collected from all recruited household members.

- Original data collection forms or database will be handled only by staff members and kept under locked storage until completely coded, checked and transported for data entry. Once data entry and cleaning are completed the original forms will be stored under lock and key at the Defeat Malaria Office until final analyses and reports have been prepared. They will then be destroyed.

8. Quality Control

8.1. Training and supervision of field teams and quality management

A field research coordinator (FRC) will be dedicated to ongoing supervision and monitoring of study implementation. Clinical procedures and data collection will be evaluated regularly by the FRC. Other quality control (QC) measures will include daily review of patient records, observation of interviews and clinical procedures, adherence to the approved protocol and Standard Operating Procedures (SOPs) and ongoing evaluation of malaria laboratory procedures according to standardized checklists. Refresher training will be provided as necessary. The PI will lead the preparation of the study together with the field team, prior to implementation, and supervise and support the field team throughout the study. Regular visits will be scheduled every 3 months and make additional support visits as needed.

The UMB-DMR collaborative team will provide training, and re-training if necessary, prior to the study initiation. Monitoring of the data and sample quality will be ongoing throughout the study, while study procedures are carried out and when the samples are delivered. Feedback, and re-training as needed, will be provided based on the observed quality of the data and sample.

8.2. Diagnostics (conventional and highly-sensitive malaria RDT)

Routine quality control procedures will include close monitoring and supervision of RDT performance against standardized monitoring checklists on a monthly basis.

8.3. Quality management of data and samples

Established procedures for monitoring and documenting the quality of both clinical and molecular data will be followed, using a protocol-specific Data Monitoring Plan. This plan will include monitoring of source documents and a subset of case record forms, systematic sliding scale evaluation of study documents, systematic assessment of the quality of DBS specimens, and an external quality assurance exercise organized by the laboratories at the University of Maryland in Baltimore to validate the results of molecular assays done at in-country laboratories.

9. Ethical Considerations

The study will be performed in accordance with ethical principles based on Declaration of Helsinki and International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP) and all applicable regulatory requirements.

9.1. Informed Consent/Assent

The principles of research ethics in the current edition of the Declaration of Helsinki will be applied, and protocol-specified procedures will be initiated only after obtaining informed consent. The written consent documents will embody the elements of informed consent as

described in the current edition of the Declaration of Helsinki, will adhere to the ICH Harmonized Tripartite Guideline for Good Clinical Practice and 45CFR46 and 21CFR50, and will also comply with applicable Myanmar regulations. The oral consent process will be consistent with 45CFR46.§46.117, 21CFR50.27 and ICH E6 (R1) Section 4.8.

Information about the study will be given to prospective participants, in a simple lay language both in Myanmar and Rakhine, in both oral and written forms whenever possible. Independent witnesses will be used to attest that illiterate potential participants have understood the contents of the informed consent document.

All informed consent, assent and adult guardian permission will be seek in local language. The written forms will be translated in to the respective local languages (Myanmar) and then translated back to English by a second translator to verify accuracy of information or certified by an official translator. These will be provided to participants and/or their caretakers in their respective local language. If a participant or parent/guardian is illiterate, the consent form will be read to them in their respective local language and a thumbprint will be accepted as a legally effective signature. In such situation a witness signature will be sought. Consenting participants and/or their caretakers will be advised that they are free to decline any question or procedure and that they may terminate their participation at any time.

All adult participants (18 years of age and above) will be asked to provide informed consent.

Children 13 to ≤ 17 years of age will be asked to provide written assent, in addition to written consent provided by their guardian.

Children 8 to ≤ 12 years of age will be asked to provide verbal assent, in addition to written consent provided by their guardian.

For children aged 5 to ≤ 7 years of age, adult guardian permission will be obtained.

The study investigators and staff will closely work with the NMCP and other relevant local research staff at the study sites, providing training, and re-training if necessary, in Protection of human subjects, Good Clinical Practice, and responsible conducts of research to assure study information and informed consent procedures meet requirements of the community, sponsor and all relevant ethic review oversight committees. UMB investigators have published detailed descriptions of the processes that have been used to obtain community “permission to enter” and individual informed consent [42]. During the consent process, emphasis will be made to ensure that the participant understand that the study participation is entirely voluntary, refusal to participate will not have any negative consequences or repercussion and free to withdraw from the study at any time. We consider informed consent to be a dynamic, ongoing process, with continuous availability of investigators to answer any questions that arise in the course of the study and to ensure that participants and their parents/guardians understand study procedures. Even though subject participation will be brief for this study, these same principles will be followed.

9.2. Confidentiality

Participant’s confidentiality is held strictly in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples, in addition to the demographic information relating to participating subjects. The study protocol, documentation, data and all other information generated will be held in strict confidence.

Study records including case record forms and consent forms will be stored in locked cabinets in secure facilities only accessible to authorized investigators.

The data generated from each study sample will be linked to the coded, unique identifier provided by study investigators on the original sample container, as well as any relevant information such as parasite counts. Study data will be stored on password protected computers. Data from this study may be submitted to publicly available databases if and when appropriate, but will not be linked to any identifying information from study participants. Participants will not be identified in any publications resulting from the study.

9.3. Vulnerable Populations

Children and other (those living in poverty, limited education or poor literacy), considered vulnerable, will be enrolled in the study. This is justified since all are at risk for malaria, all must be included in malaria surveillance, and children may have a different parasitological profile than adults. The study team will undergo proper training in human subject protection and good clinical practice to understand ethical issues related to conducting research in vulnerable populations.

No justification for waiver or alteration of informed consent is being sought.

9.4. Potential Risks and Benefits and Procedures to Minimize Risks

Risks: There are risks of bruising, bleeding, infection and fainting from blood collection, of potential loss of confidential and private information. There are also risks to study personnel such as accidental exposure to blood and body fluid borne infections. We will take proper procedures to minimize all the risk. We will provide protocol-specific didactic and hands-on training including good clinical practice and human subject protection prior to the study initiation, careful evaluation during the early stage of the study, and re-training based on our evaluation as needed. Standard Operating Procedures (SOPs) for staff safety are used in clinical and laboratory areas, including sharps management, hazardous waste management, universal precautions are used for handling all body fluids. Since no sensitive information will be collected, social or economic risks are considered low.

Benefits: Participants may not directly benefit from their participation in the study. However, if symptoms of malaria or other illnesses are noted participants, they will be referred for evaluation and for prompt treatment at the appropriate local clinic. There will be benefits to the community could include improved tools for surveillance and diagnosis of antimalarial drug resistant malaria. The finding of the study will be an important input for the national malaria control program in Myanmar as they develop the strategies to conduct RCD.

We believe that the study poses minimal appreciable additional risks to the study participants, but may provide potential benefits to their communities and others living in malaria endemic areas, and the risk-to-benefit ratio is acceptable and justifiable to conduct this study.

9.5. Conflict of interest

The study team members have been trained on the conflict of interest, and none of the study team members has declared any financial or other conflicts.

10. Cooperation with national and local partners

We have been working with and will continue to seek guidance from the Myanmar national, regional and township administrative and technical leadership including, but not limited to, the regional and township administration, Ministry of Health and Sports, Vector-borne Disease Control, National Malaria Control Program, Township Medical Services and related networks. We will seek appropriate administrative and ethical approval from all relevant review committees in Myanmar and in the USA before the initiation of the study. We will hold initial meetings led by the NMCP and organized with all partners involved in the research (NMCP, VBDC Rakhine, DMR, Defeat Malaria partners, local authorities and community leaders) to explain the nature and scope of the study, and prompt their active collaboration, and maintain sufficient communication and information flow throughout the study.

11. Result Dissemination, Data Publication and Authorship

After the completion of all data analysis, preliminary data will be presented and discussed with the relevant Myanmar leadership, VBDC, NMCP, Defeat Malaria partners and the development partner. A final dissemination workshop will be organized to present and discuss the results to a large audience involving all national and international organizations involved in malaria control and elimination in Myanmar.

Findings and data will be used to indicate the possible role of this hsRDT in the RCD strategy during the elimination phase, and in the development of national SOPs on active case detection. Publication of the results in a peer-reviewed journal will be considered.

Data generated from this study are highly valuable for the whole public health community, particularly for those deeply involved in malaria elimination, and timely and prompt dissemination of data (before peer-review publication which may take a long time) is critical. After proper review, discussion and getting approval from the country's leadership and the development partner, we will share the data in several venues (USAID/PMI databases, web-based media) with known good accessibility by a large public, as well as with international agencies such as WHO and USAID/CDC. Together with NMCP, URC and DMR, the UMB team will provide a lead role in data analysis, interpretation and preparation and publication of manuscript, and presentation of data in international public health arena such as the annual meeting of American Society of Tropical Medicine and Hygiene. Authorship will be discussed and decided collectively based on the multiple means of contribution to the success of the study.

12. Timeline

Activity	2017								2018			
	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Proposal development												
Ethical submission												
Training/advocacy/Pilot testing												
Data collection												
Data entry/ analysis												
Report writing												
Dissemination of results												

REFERENCES

1. Chen I, Clarke SE, Gosling R, Hamainza B, Killeen G, Magill A, O'Meara W, Price RN, Riley EM: "Asymptomatic" Malaria: A Chronic and Debilitating Infection That Should Be Treated. *PLoS Med* 2016, 13:e1001942.
2. malERA Consultative Group on Drugs: A research agenda for malaria eradication: drugs. *PLoS Med* 2011, 8:e1000402.
3. Stephens JWW, Christophers MB: The Malarial Infection of Native Children. In *Reports to the Malaria Committee of The Royal Society*. pp. 2-14: The Royal Society; 1900:2-14.
4. Adams M, Joshi SN, Mbambo G, Mu AZ, Roemmich SM, Shrestha B, Strauss KA, Johnson NE, Oo KZ, Hlaing TM, et al: An ultrasensitive reverse transcription polymerase chain reaction assay to detect asymptomatic low-density *Plasmodium falciparum* and *Plasmodium vivax* infections in small volume blood samples. *Malar J* 2015, 14:520.
5. Cheng Z, Wang D, Tian X, Sun Y, Sun X, Xiao N, Zheng Z: Capture and Ligation Probe-PCR (CLIP-PCR) for Molecular Screening, with Application to Active Malaria Surveillance for Elimination. *Clin Chem* 2015, 61:821-828.
6. Okell LC, Ghani AC, Lyons E, Drakeley CJ: Submicroscopic infection in *Plasmodium falciparum*-endemic populations: a systematic review and meta-analysis. *J Infect Dis* 2009, 200:1509-1517.
7. Imwong M, Hanchana S, Malleret B, Renia L, Day NP, Dondorp A, Nosten F, Snounou G, White NJ: High-throughput ultrasensitive molecular techniques for quantifying low-density malaria parasitemias. *J Clin Microbiol* 2014, 52:3303-3309.
8. Adams M, Joshi SN, Mbambo G, Mu AZ, Roemmich SM, Shrestha B, Strauss KA, Johnson NE, Oo KZ, Hlaing TM, et al: An ultrasensitive reverse transcription polymerase chain reaction assay to detect asymptomatic low-density *Plasmodium falciparum* and *Plasmodium vivax* infections in small volume blood samples. *Malar J* 2015, 14:520.

9. Imwong M, Nguyen TN, Tripura R, Peto TJ, Lee SJ, Lwin KM, Suangkanarat P, Jeeyapant A, Vihokhern B, Wongsan K, et al: The epidemiology of subclinical malaria infections in South-East Asia: findings from cross-sectional surveys in Thailand-Myanmar border areas, Cambodia, and Vietnam. *Malar J* 2015, 14:381.
10. Searle KM, Shields T, Hamapumbu H, Kobayashi T, Mharakurwa S, Thuma PE, Smith DL, Glass G, Moss WJ: Efficiency of household reactive case detection for malaria in rural Southern Zambia: simulations based on cross-sectional surveys from two epidemiological settings. *PLoS One* 2013, 8:e70972.
11. van Eijk AM, Ramanathapuram L, Sutton PL, Kanagaraj D, Sri Lakshmi Priya G, Ravishankaran S, Asokan A, Tandel N, Patel A, Desai N, et al: What is the value of reactive case detection in malaria control? A case-study in India and a systematic review. *Malar J* 2016, 15:67.
12. Hustedt J, Canavati SE, Rang C, Ashton RA, Khim N, Berne L, Kim S, Sovannaroth S, Ly P, Menard D, et al: Reactive case-detection of malaria in Pailin Province, Western Cambodia: lessons from a year-long evaluation in a pre-elimination setting. *Malar J* 2016, 15:132.