# CHAPTER II

# PROPOSAL

## Introduction

Malaria burden in reality is much worse than statistical figures that reported by WHO each year approximately 1 million deaths in Africa alone[1]. The limitation of diagnosis is a major contribution, which affect to the prevalence of the disease.

In malaria endemic areas, the clinical features alone cannot provide information of malaria diagnosis. Therefore, clinical diagnosis is not only useless but also urges for drug resistance especially in *P. falciparum* infection. To be success in malaria control, a laboratory diagnosis and prompt treatment are needed.

In rural areas, laboratory diagnosis for malaria is difficult to assess because it requires well-trained and experienced laboratory technician, careful and good techniques, regularly microscopy maintenance, logistics demanding and the most important is a regular quality control.

Regarding to the above constraints, Rapid Diagnostic Tests are considered interesting whether to supplement or to replace the microscopy. Hence, this proposal proposes the performance evaluation of the OptiMAL Rapid Malaria Test: OptiMAL-IT (Flow Inc., Portland, OR,) compared to microscopy as the gold standard of malaria diagnosis.

## Research Questions

**Primary research question**

- Can OptiMAL-IT be used to supplement or replace microscopy for malaria diagnosis in the peripheral health system?

**Secondary research question**

- What is the performance of the diagnostic characteristics of the new generation of OptiMAL Rapid Malaria Test: OptiMAL-IT and the comparison between OptiMAL –IT and Paracheck Pf?

## Research Methodology

**General objectives**

- To evaluate the diagnostic characteristics of the new generation of OptiMAL Rapid Malaria Test: OptiMAL-IT
- To evaluate the diagnostic characteristics of the HRP-2 capture dipstick: Paracheck Pf

➢ **Specific objectives**

- To assess the Sensitivity, Specificity, PPV, NPV of OptiMAL-IT

**Sensitivity**-Percent of diseased persons who test positive

**Specificity-Percent of non-diseased persons who test negative**

**Positive predictive value (PPV)**-Percent of positive tests that are truly positive

**Negative predictive value (NPV)**-Percent of negative tests that are truly negative

- To compare the above parameters between the current Rapid Diagnostic Test used in the field (Paracheck Pf) and **OptiMAL-IT.**

- **Hypothesis**

None

- **Assumption**

  - The study was blinded the lab technicians performing OptiMAL-IT, Paracheck-Pf and microscopy at 1st and 2nd level did not know the results of each others.
  - The results of the microscopy considered as a gold standard.

- **Study design and variables**

  - Cross-sectional descriptive study
  - Independent variables were the various level of parasitaemia.
  - Dependent variables were diagnostic characteristics of OptiMAL- IT and Paracheck-Pf test.

- **Study site**

The Thai-Karen village called Ban Maw Ker Thai, located on the Moei river bank, Pop Phra district, Tak province, Thailand.

- **Methods**

**Criteria for the exclusion in the study**

- **Children under 2 years of age.**

There was a requirement of 250 µL of blood sample therefore to take sample from children under 2 years of age was difficult.

- **Pregnant women**

The effect of pregnancy to the level of pLDH is not clear, so I decided to exclude the pregnant women from the study.

- **Patient with severe sign of malaria infection**

There were many steps in the study procedure such as registration, Paracheck-Pf test, OptiMAL test, Hematocrit test and smearing so the patient who came with the sign of severity deserve the proper treatment in time so they were excluded from this study.

**Criteria for the inclusion in the study**

Outpatients with clinical symptoms, of malaria: fever and/or history of fever in the past 48 hours plus one or more of the following symptoms: headache, joint/muscle pain, abdominal pain, attending the Ban Mor Ker Thai clinic.

A written consent in the patient's own language will have to be obtained prior to admission. (Appendix; Consent form).

**Definitions of blood sample/Description of the sample collection procedure:**

On admission:

Finger prick for: Blood slides (combined slide: thin & thick smear);

(2 sets of slides)

OptiMAL and Paracheck tests and Hematocrit test.

**Sample size:**

Sample size calculation were done as follows:

Given an expected sensitivity of a test of 90%

An alpha error of 0.05,

The number of blood smear positive needed to achieve a precision of 5%

$$N = Z^2pq/d^2$$

N = Number of positive sample

Z = 1.96 at alpha = 0.05

p = Sensitivity of instrument

q = 1-p

d = Alpha error

Therefore the number of blood smear positive was 136. That is to say a total number of about 150 positive people. The expected specificity was fixed at 90% with an alpha error of 0.05. The number of blood smear negative people needed to achieve a precision of 5% was 136. That is to say a total number of about 150 negative people. In conclusion, a total number of 300 persons.

## Data Collection, Entry, Coding and Analysis:

Clinical and demographic data were recorded as routine procedure. Results of OptiMAL-IT and Paracheck Pf and microscopic examination were recorded in seperate forms to keep the laboratory technician unaware of the OptiMAL-IT and Paracheck Pf results. Data were entered in a database and analysed using Statistical Package for Social Science (SPSS) computer software package. After data entry, the database was entirely checked against the source documents, and errors were corrected. The baseline data were summarized for descriptive statistics in term of frequency, percentage, mean, standard deviation and etc. The results of the study were analyzed to establish the performances of OptiMAL-IT and Paracheck Pf test compared to the gold standard (microscopy thick and thin blood films). The sensitivity, specificity and PPV and NPV with 95% Confident Interval were tabulated. These OptiMAL-IT test compared to the

gold standard (microscopy thick and thin blood films). The sensitivity, specificity and PPV and NPV were tabulated. These parameters were compared between HRP-2 capture dipstick (Paracheck Pf) and OptiMAL-IT by using $\chi^2$ statistics and p values< 0.05 were indicated of statistically significant differences. The analysis took into account the various level of parasitaemia.

## Definition of the Methods/Reference Method Used

**Method-1: Microscopy**

**1st level** (dispensary-lab in the Ban Mor Ker Thai village)

2 slides combining thin smear (half-slide) and thick smear (the other part of the slide) (Appendix; Microscopy form I )

**Microscopy reading**

(Appendix; Microscopic examination procedure)

200 fields for thick smear parasitaemia were counted against 500 WBC; if parasite count > 500/500 WBC the count was expressed in % of infected RBC on thin smear.

The classification of parasitaemia corresponded to the latest published reference by A. Moody ( Clin.Microbio.Rev, 15(1), 66-78, 2002).

The following published range of parasitaemia had to be used (adaptation for the last group):

| | | |
|---|---|---|
| Group-1: | More than 1% | (i.e.50,000 parasites/μL) |
| Group-2: | Between 0.1-1% | (i.e.between 50,000 and 5000 parasites/μL) |
| Group-3: | Between 0.1-0.01% | (i.e.between 5,000 and 500 parasites/μL) |

Group-4: Between 0.01-0.001% (i.e.between 500 and 100 parasites/μL)

Group-5: Less than 0.002% (i.e.less than 100 parasites/μL)

**2nd level**:( Quality control protocol in the Shoklo Malaria Research Unit laboratory of Mae Sot) (Appendix; Microscopy form II)

Following parameters had to be noted:

a) Microscope magnification: objective and ocular lens

b) Staining procedure (fresh 10% Giemsa solution, 20 minutes)

c) Observation time for 200 fields

**Method-2: OptiMAL-IT test and Paracheck-Pf test**

The OptiMAL dipstick test and Paracheck-Pf were performed according to the manufacturer's instruction. (Appendix: OptiMAL leaflet and Paracheck Pf leaflet)

A special result report form had to be used in order to collect information about the final result: - or +, and their interpretations for *P.falciparum* or for *non-P.falciparum (P.vivax, P.ovale, P.malariae)* (Appendix ; OptiMAL-IT result form)

A special result report form had to be used in order to collect information about the final result: - or +, and the intensity of positive result in *P. falciparum* infections. (Appendix; Paracheck Pf result form)

## Report of the Results

If there is a difference of a result between the reference method and **OptiMAL-IT**, the microscope slide and the OptiMAL-IT test strip should be kept and be made available for verification.

## Ethical Consideration

The ethical committee of the College of Public Health, Chulalongkorn University before beginning would approve the study. All of the participants were inform consent before recruiting in the study.

## Expected Obstacles

- **Security situation**

There was ongoing of fighting on the Burmese site near Ban Maw Ker Thai, this affected to the patients that they could not be able to travel or came to the study site.

- **Rain and flooding**

There was a heavy rain during the rainy season, a malaria transmission season which affected to transportation and traveling. The flooding increased the Moei river higher, people and boats could not cross the river to Ban Mor Ker Thai clinic.

## Study Plan

The study plan was started in February 2002 and was finished by October 2002. A brief description was shown in table below.

**Table 2: Activities plan for OptiMAL-IT evaluation study**

| Activity | Feb | Mar | Apr | May | Jun | Jul | Aug | Oct |
|---|---|---|---|---|---|---|---|---|
| • Proposed the study to DiaMed & cooperated for the OptiMAL shipment | √ | | | | | | | |
| • Designed the study protocol and reviewed the previous studies | | √ | | | | | | |
| • Revised the protocol | | | √ | | | | | |
| • Q.C. for microscopy in Maw Ker Thai team | | | √ | | | | | |
| • Informed the staffs for the study<br>• OptiMAL and Paracheck Pf training to staffs<br>• Designed the blinded study in Ban Maw Ker Thai<br>• Practiced the data collection in Ban Maw Ker Thai | | | | √<br>√<br>√<br>√ | | | | |
| • Data collection at Ban Maw Ker Thai | | | | | √ | √ | | |
| • Analysis | | | | | | | √ | |
| • Report writing & conclusion | | | | | | | | √ |

## Budget

The buget required to provide financial support for OptiMAL –IT study in rural Thai village. The estimate expenditure for study activities was shown in table below.

**Table 3: Estimate expenditure for study activities**

| Budget catagory | Unit cost (Baht) | Multiplying factor | Total cost (Baht) | % of Total |
|---|---|---|---|---|
| **Personnel** | | | | |
| • Microscopy lab technicians for on site laboratory. | 200 per day | 2 persons x 21 days | 8,400 | |
| • Microscopy lab technicians for Mae Sod laboratory | 200 per day | 2 persons x 21 days | 8,400 | |
| • Medic at registration | 200 per day | 1 person x 21 days | 4,200 | |
| • Paracheck-Pf staff | 200 per day | 2 person x 21 days | 8,400 | |
| • OptiMAL staff | 200 per day | 2 person x 21 days | 8,400 | |
| • HCT staff | 200 per day | 1 person x 21 days | 4,200 | |
| • Professional trainer for staff training | 500 per day | 1 person x 1 days | 500 | |
| • Driver to Ban Maw Ker Thai | 200 per day | 1 person x 51 days | 10,200 | |
| **Total** | | | **52,700** | **60%** |

**Table 3: Estimate expenditure for study activities (Cont.)**

| Budget catagory | Unit cost (Baht) | Multiplying factor | Total cost (Baht) | % of Total |
|---|---|---|---|---|
| **Logistics**<br>• OptiMAL Tests | 80 per test | 400 tests x 80 Baht | 32,000* | ***Supported from DiaMed** |
| • Paracheck-Pf Tests | 25 per test | 300 tests x 25 Baht | 7,500 | |
| • Microscopy supplies | 15 per test | 300 tests x 15 Baht | 4,500 | |
| **Total** | | | **12,000** | **14%** |
| **Transportation & fuel** | 250 per day | 21 days x 250 Baht | **12,750** | **15%** |
| **Miscellineous and supplies** | - | - | **10,000** | **11%** |
| **Grand total** | | | **87,450** | **100%** |

# References


Joel G. Breman 2001. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *American Journal of Tropical Medicine and Hygine* 64,1-2S :1-11.

Moody A. 2002 Rapid Diagnostic Tests for Malaria Parasites. *Clinical Microbiology Reviews* 15, 1 (Jan) : 66-78.