VALIDATION OF MICROSCOPE EQUIPPED WITH A VERSATILE ILLUMINATOR (THE EARL-LIGHT) IN DETECTING MALARIA PARASITES

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ABSTRACT: Some malaria endemic areas of Thailand are remote areas having no electricity supply and need more competence device than natural light microscopy to improve malaria case detection. This study was to validate the microscope equipped with a versatile illuminator (the EARL-Light) in detecting malaria parasites. The study was conducted as a cross-sectional study to examine 800 field malaria suspected cases at Mae-Sod District, Tak Province, Northern Thailand. Thick blood films were examined by field technicians using either monocular or binocular microscopes equipped with the Earl Light illuminator compared to sunlight microscopes. Their results were evaluated with the results from experienced microscopists at the Laboratory Reference Center for Vector Borne Disease and the validities were evaluated. The result showed that the sensitivity of Earl Light equipped with binocular microscope was quite superior over that of the natural light one whereas this superiority was not obviously shown for monocular microscope. The major factor might be due to the experience in malaria diagnosis and unaccustomability of the users to this tool. The tool could use one pack of 4 A batteries for half a month. Most of the experimental users felt advantage to this tool but minors needed to improve the tool's attribution. The tool would give some sort of extremely benefit to strengthen the routine diagnostic method to detect malaria cases in non electricity remote areas and binocular microscope equipped with this device is preferred to the monocular microscope. In addition, further validating study is needed to confirm for large scale applying in any malaria control program.

Keywords: malaria diagnosis, monocular microscope, binocular microscope, the EARL Light illuminator

INTRODUCTION: Malaria, especially *Plasmodium falciparum*, is recognized as a serious health problem in tropical and subtropical regions of the world and one that has far-reaching medical, social and economic consequences for the countries in which it is found. It also causes high mortality. Recently, World Health Organization reported that the incidence of malaria in the world may be on the order of 350-500 million clinical cases each year with countries in Tropical Africa accounting for more than 80 % of the world's cases. Of the non-Africa cases, 90% came from India, Brazil, Sri Lanka, Afghanistan, Thailand, Viet Nam and Colombia[1)].

In Thailand, malaria was the deadly disease, producing 297.1 deaths per 100,000 population in 1974, decreased to 7.8 per 100,000 population in 1982, and 0.36 per 100,000 population in 2004. The morbidity rate in 1974 was 286 per 1000 populations, with gradual decrease thereafter during 1988 - 2002 at 6.71 to 0.82 per 1,000 populations. In 2004 the prevalence was slightly decreased with API (Annual Parasite Incidence) of 0.51 per 1,000 population[2)].

Persistence of malaria incidence in Thailand was due mainly to population movement and parasite resistance to drugs. The latter has probably become the most important threat to effective control of the disease. The other major factors are probably due to some minor epidemics take place along the Thai-Myanmar border due to influx of minority groups, occupational migration and unstable political situation. Likewise in other remote areas, especially in moderate transmission areas, the same factors provoking malaria are appealed including the influx of non-immune travelers, refugees, woman during pregnancy, displaced persons and laborers. They will mostly become severe malaria symptoms if they get infection. The routine malaria diagnosis, Giemsa

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thick blood film examination (TBF), is still the best technique now available. TBF has been satisfactory in term of sensitivity and specificity for the diagnosis of malaria. However, the examination has often been hindered by the lack of a reliable light source to illuminate the specimens. In most peripheral levels of the primary health care system, which do not have electricity supply, natural light source may not be enough to illuminate the microscopes to look for the parasites. Besides, in the areas where have electricity supply but during power failures, microscopes which depend on a light bulb are unusable. Microscopes equipped with a mirror that reflects ambient daylight are also unusable at night, during cloudy days, or when far from a well-lighted window[3].

Many studies were conducted to validate microscopical diagnosis at the peripheral health care level. Coleman, *et al.* found that field microscopy gave specificities of 99.3 % and 99.2 % for the diagnosing *P. falciparum* and *P. vivax* malaria respectively, in those samples that mainly consisted of parasitemia less than 500 parasites/ microlitre. Nevertheless, the sensitivity was only 10.0 % and 7.1 % respectively[4]. This study was discordant from the finding of Bualombai, *et al.* that field microscopy gave 85.7 % and 57.4 % sensitivity for diagnosing *P. falciparum* and *P. vivax* respectively. However, the specificity was very high (more than 98 %) as well[5].

New innovative methods are extremely needed to improve diagnostic performance at the peripheral health care levels especially in the remote areas which do not have electricity. In those areas, monocular microscopes have been normally used with 700X–1000X in magnification and sunlight as the light source. Barring those areas, some often use binocular microscopes with sunlight as the light source, despite the fact that the binocular microscope should be efficiently used with tungsten bulb. Insufficiency of light illumination hinders the microscopist performance.

Most recently, there is a promising versatile illuminator initially developed by the US Centers for Disease Control and Prevention (CDC, Atlanta, USA). This tool, the EARL light, could be equipped with monocular and binocular microscopes and applied to detect malaria parasites in the remote areas of Thailand. This study aimed to compare malaria diagnostic values of TBF field examinations between using the EARL–Light illuminator and sunlight as the light sources. Both diagnostic values examined by the same field technician were validated by comparison with TBF examined by experienced microscopists. User acceptance and satisfaction were also determined.

MATERIALS AND METHODS:

Selective areas and samples

Mae Sod district, Tak province located in the West-Northern region of Thailand was purposely chosen because it has been a hyperendemic area with API more than 10 per 1,000 population[2]. The areas lie south west of the Myanmar border. The average malaria species formula in 2004 was 49.3% *P. falciparum*, 48.1% *P. vivax*, 0.11% *P. malariae* and 0.51% mixed infection (Pf. + Pv.). Health care is provided by 8 malaria clinics, and 30 health centers, district hospital and a system of village volunteers. The areas have multi-resistant *P. falciparum* status. Many areas in this district are remote non-microscope areas. Mobile malaria clinics are normally set up during high transmission period (during May-October).

The number of patients was calculated using Dr. Douglas Tang's technique applied from Machin and Campbell in 1987[6], and Dixon and Massay in1983[7]. The calculation of the sample size was based on the estimation of approximately 98 % sensitivity and .02 specified width of 95 % confidence interval (lower, 1-sided). Then, 794 cases were gained by opening the reference table to rule out the sensitivity equal or less than 95 % with 90 % Power (1-β).

Five apart mobile malaria clinics in Tak district were purposely chosen, as follow: Amphoe Muang Maesod, Ban Mae Ku, Ban So O, Ban Phop Phra, and Ban Valae.

Patients were interviewed and/or observed for the following inclusion / exclusion criteria:

Inclusion criteria:

1) at least one year olds
2) Having oral temperature > 38° C, headache or history of fever within 72 hrs.
3) Having history of entering malaria transmission areas during the past 1 month

4) Having parasitemia less than 2,500 parasites/ 1,000 WBC
5) Written informed consent

Exclusion criteria:

1) Pregnant
2) Having parasitemia higher than 2,500 parasites/ 1,000 WBC

The parasite count (per microlitre) was calculated following the method described elsewhere[8].

Diagnostic techniques

The EARL-Light illuminator uses three flashlight batteries which have been reported previously to provide power for 1,250 hours (one year of work, at 4 hours a day, 6 days per week). The device composes of battery pack, power light-emitting diode with flexible ingot which allows the microscopist to direct the light beam towards a microscope mirror or to shine directly into a microscope condenser. Moreover, the light-emitting diode produces an intense, cool bluish white light thus no filter required and no heat produced. This tool has been used to improve the detection of many infectious diseases such as Tuberculosis and Malaria under field conditions especially at peripheral health care units.

Microscopic method, thick and thin blood films were made and stained with 10 % Giemsa stain. The thick blood films were read strictly by 100 microscopic fields (visible microscopic fields on blood film by looking through 1000 X microscope), before interpreting the films' results. When a malaria parasite was found in the film, it was counted against 500 white blood cell (WBC), or 5,000 red blood cells (RBC) in the thick and thin blood films respectively. Thereafter, thick and thin blood films were reexamined and parasitemia recounted independently by two expert micro-scopists at the Laboratory Reference Center for Vector Borne Disease, Bureau of Vector Borne Disease, Nonthaburi, Thailand. The results from both microscopists should be given concordance. However, in case some blood films give discordance, the blood films must be reexamined by third expert microscopist, and the blood film results obtained in this step were adopted as the gold standard for field diagnostic validations. For all cases, parasitemia enumerated per μl of blood as the average of two slide reading results based on hematologic estimates of 7,500 WBC/ μl and 500,000 RBC/μl of blood.

Experimental trial

This study was a complete blinding study. At the time of field trial, the patients were explained thoroughly about the project and they must have agreed to participate in the study by filling the consent form designed by the Ethical Committee of the Department of Disease Control. Then, the patient was routinely blood filmed by the malaria clinic staffs for microscopical diagnosis. Two thick and one thin blood films were made per each patient. Thick blood film was used to identify parasite; meanwhile, thin blood film was used to confirm malaria species, in case any microscopists were unable to recognize the parasite species. The blood films were stained and then examined by local technicians using the standard documented operating procedures, under 1000 X power oil – immersion - field. The technicians were blinded for the subject's medical status. Of the two thick blood films, one was sent to the laboratory for standard examination as mentioned above. The other was recoded and examined by the first set of field technicians using monocular microscope with sunlight as light source. As soon as diagnosis finished, each film was recoded again and was reexamined by the same technician using the microscope equipped with the Earl Light illuminator. After that, the second trial was performed in the same way by the new set of technicians but using binocualar microscope instead. All malaria positive patients were treated by following the local protocol and guideline in accordance with the national guideline by the Bureau of Vector Borne Disease.

The technicians were interviewed promptly after works about their acceptance and satisfaction with the EARL-Light illuminator.

Data analysis

The diagnostic values consisting of sensitivity, specificity, accuracy, predictive value and inter-rater reliability (Kappa) were determined using SPSS Statistical Software Program Version 14.

RESULTS: Ten local technicians were trained at the Center for Vector-Borne Diseases, Maesod district, Tak province on June 03 and 10, 2007. As soon as the training was finished, they went back to 5 experimental Malaria Clinics for preparing the field studies in the next day. One hundred and sixty malaria suspected cases, were collected in each malaria clinics on the first three days of the experiment and the total number of samples was 800 cases.

On the first three days, the first set of technicians who used monocular microscope to diagnose the blood films, examined 800 blood films by using natural light source. Then, as soon as the examination finished, the 800 blood films were recoded and subsequently examined again by using the monocular microscope equipped with the EARL Light illuminator (Figure 1). Table 1 showed the comparison between the blood film results of malaria parasite species detected by monocular microscopy with natural light source and Earl Light illuminator.

All 800 blood films were confirmed by experienced microscopists, the results were used as gold standard. The diagnostic values of either natural light or EARL Light microscopy compared to gold standard were described in table 2. In addition, the 800 blood films were re-diagnosed by binocular microscopes equipped with either natural light or Earl Light illuminator respectively. The diagnostic values compared to gold standard were shown in Table 3.



Figure 1 A field technician at Phop Phra Malaria Clinic examined his blood film by using monocular microscope equipped with Earl Light Illuminator.

Table 1 Comparison of malaria parasite diagnosis by monocular microscopy using natural light and the EARL Light illuminator

The Earl Light monocular microscopy	Natural light monocular microscopy					Total
	F	Fg	V	F+V	N	
F	35	0	0	0	1	36
Fg	0	0	0	0	1	1
V	2	0	54	0	1	57
F+V	0	0	0	0	0	0
N	1	1	1	0	703	706
Total	38	1	55	0	706	800

F= *P. falciparum*, Fg = *P. falciparum* gametocyte, V= *P. Vivax*, F+V = mixed infection consisting *P. falciparum* and *P. vivax*, N= Negative

The sensitivity of each type of microscopy classified by parasitemia was shown in Table 4.

Interviewing for user acceptance and satisfaction showed that most technicians agreed that the Earl Light brightened more than natural light. When asked about how contrast affected in differentiating the chromatin and chromosome of the malaria

Table 2 Diagnostic values of TBF field examination using natural light (A) or Earl Light illuminator (B) equipped with monocular microscopy related to experienced microscopist examination

A. Natural light - monocular microscopy

Expert microscopy result	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV* (%)	NPV** (%)	Kappa
Total	84.1	99.0	97.0	92.8	97.6	0.848
P. falciparum	83.3	99.2	98.4	81.1	99.2	0.790
P. vivax	78.8	99.5	97.6	93.3	98.0	0.842

B. EARL Light illuminator - monocular microscopy

Expert microscopy result	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV* (%)	NPV** (%)	Kappa
Total	88.2	98.6	96.4	89.8	97.3	0.816
P. falciparum	80.6	98.6	97.8	72.5	99.1	0.731
P. vivax	76.1	99.5	97.4	93.1	97.7	0.823

* PPV, positive predictive value **NPV negative predictive value

Table 3 Diagnostic values of TBF field examination using natural light (A) or Earl Light illuminator (B) equipped with binocular microscopy related to experienced microscopist examination

A. Natural light - binocular microscopy

Expert microscopy result	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV* (%)	NPV** (%)	Kappa
Total	76.6	98.4	95.5	88.2	96.5	0.784
P. falciparum	52.8	99.2	97.1	76.0	97.8	0.295
P. vivax	84.5	98.9	97.6	88.2	98.5	0.421

B. EARL Light illuminator - binocular microscopy

Expert microscopy result	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV* (%)	NPV** (%)	Kappa
Total	85.1	99.0	97.1	92.9	97.7	0.854
P. falciparum	72.2	99.1	97.9	78.8	98.7	0.226
P. vivax	85.9	99.5	98.3	93.9	98.6	0.444

* PPV, positive predictive value **NPV negative predictive value

parasite, 8 in 10 of them said that the illuminator gave more contrast than natural light. But the rest said it flashed intermittently to their eyes. Furthermore the tool's light control ingot allowed them to adjust the light only two levels, dim and bright. They recommended that the tool should be improved its light control knob to increase more steps of lighting from dim to full bright. In addition, one user complained about double vision. For the duration of illuminating, nine users said that the tool had well illuminating through their examination time and ensured that the tool maintained its consistent well lighting more than 20 hours. Nine of ten users preferred this tool to the conventional one. However, four of ten said that the tool was more complicated than the conventional sunlight microscope whereas the others felt like they routinely worked every day. Finally, nine of ten technicians ensured that this illuminator could be an auxiliary part of the natural light microscope particularly in remote endemic areas and they also ensured that the tool would increase the capacity of malaria case detection in any malaria control program.

DISCUSSION: Trying to find out the developed diagnostic assay in non-electricity supply remote area having only natural light microscope is very tough. A versatile illuminator (the EARL-Light) could be equipped with the conventional microscope and expected to solve this problem. This study was to diversify the illuminator to fortify the sunlight microscopic field diagnosis.

According to Table 1, monocular microscopic examination was compared between using sunlight and illuminator. The results showed 99% coincidence between these 2 light sources (35 *P. falciprum*, 54 *P. vivax* and 703 negative from total 800 specimens). However, there seemed to be no absolutely superior between them.

Table 4 Sensitivity of the four experiments, at different level of parasitemia in field study patients.

Parasitemia/µl	Sensitivity (%)			
	NM	EM	NB	EB
1-500	60	60	50	50
501-1,000	75	77	57	64
1,001-2,000	83.3	83.3	80.9	85.7
2,001 and up	92.3	92.2	89.9	94.7

NM, Natural light monocular microscopy
EM, EARL Light illuminator monocular microscopy
NB, Natural light binocular microscopy
EB, EARL Light illuminator binocular microscopy

According to the comparison of diagnostic values between either sunlight or the Earl Light microscopic examination by field technician and experienced microscopist, the EARL Light showed little superior sensitivity over natural light in case of monocular experiment and showed quite superior sensitivity over the natural light in case of binocular experiment (Table 2 and 3). The sensitivity was improved, in four experiments, when parasitemia increased (Table 4). In addition, the performance of malaria diagnosis heavily relies on individual microscopist experience; however, this study recruited the 10 microscopists which had equally working year experiences.

The EARL Light monocular microscopy gave 16 false negative and 7 false positive results when detected total parasites. The same patterns were

shown when specified the sensitivity to *P. falciparum* or *P. vivax* alone as well. But in binocular experiment, the EARL Light showed less number of false negative cases than that found in the monocular experiment. Therefore, it seemed to be that the EARL light binocular microscopy could detect more parasite than sunlight binocular, natural light monocular or EARL Light monocular microscopy. Tracking this research result, all microscopists were interviewed and found some evidences to explain the EARL Light's competence. Most of the microscopists felt advantageous to this tool except some minor limitations such as intermittently flashing, double vision and narrow brightness control. By this study, there were two recommendations; firstly, further studies should be conducted to confirm this tool's competence. Secondly, the tool equipped with binocular microscope to be used in non-electricity supply remote areas should be preferable.

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