

COMPARISON OF FIELD AND EXPERT LABORATORY MICROSCOPY FOR ACTIVE SURVEILLANCE FOR ASYMPTOMATIC *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN WESTERN THAILAND

RUSSELL E. COLEMAN, NONGNUJ MANEECHAI, NATTAWAN RACHAPHAEW, CHALERMPOK KUMPITAK, R. SCOTT MILLER, VIRAT SOYSENG, KRONGTHONG THIMASARN, AND JETSUMON SATTABONGKOT

Departments of Entomology and Immunology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Sankhlaburi District Malaria Clinic, Kanchanaburi Province, Thailand; Malaria Division, Department of Communicable Disease Control, Ministry of Public Health, Nonthaburi, Thailand

Abstract. Microscopy of Giemsa-stained thick and thin films by a skilled microscopist has remained the standard laboratory method for the diagnosis of malaria. However, diagnosis of malaria with this method is problematic since interpretation of results requires considerable expertise, particularly at low parasite levels. We compared the efficacy of “field” and “expert laboratory” microscopy for active surveillance of *Plasmodium falciparum* and *P. vivax* in western Thailand. Field microscopy consisted of an approximately five-minute read (50–100 fields) of a thick film at $\times 700$ using a natural light source, whereas expert laboratory microscopy consisted of a 20-minute read (number of parasites per 500 leukocytes) at $\times 1,000$ using a high-quality, well-maintained microscope with an artificial light source. All discordant and 20% of concordant results were cross-checked blindly. A total of 3,004 blood films collected between May and November 2000 were included in the study, of which 156 (5.2%) were positive for *P. falciparum*, 177 (5.9%) for *P. vivax*, and 4 (0.1%) for both *P. falciparum* and *P. vivax* by expert microscopy. A total of 84.4% (135 of 160) of the *P. falciparum*-positive slides and 93.9% of the *P. vivax*-positive slides had a parasitemia of less than 500/ μ L. Field microscopy was specific (99.3%) but not sensitive (10.0%) for the diagnosis of *P. falciparum* malaria, with a positive predictive value (PPV) of 43.2% and a negative predictive value (NPV) of 95.1%. The corresponding specificity and sensitivity for the diagnosis of *P. vivax* malaria were 99.2% and 7.1%, respectively, with a PPV of 38.7% and an NPV of 93.9%. Field microscopy, as defined in this study, is not an effective method for active malaria surveillance in western Thailand, where prevalence and parasitemia rates are low.

INTRODUCTION

Microscopy of Giemsa-stained thick and thin films by a skilled microscopist has remained the standard laboratory method for the diagnosis of malaria.^{1–4} However, microscopy cannot be considered a “gold-standard” for malaria diagnosis because low-level parasitemias and mixed infections are frequently not detected, interpretation of results is often ambiguous, and procedures for preparation of slides and enumeration of parasites are inconsistent.³ In western Thailand, as in many other regions where malaria is endemic, a variety of problems make microscopic diagnosis, particularly at the periphery of the health care system, difficult.⁵ These include lack of skilled microscopists, limited supplies, inadequate maintenance of microscopes and reagents, and inadequate quality control. As part of a longitudinal study on vivax and falciparum malaria in western Thailand, we had an opportunity to compare the efficacy of “field” microscopy with “expert laboratory” microscopy as a means of conducting active malaria surveillance.

MATERIALS AND METHODS

Study site. The study was performed from May to November 2000 in the village of Ban Kong Mong Tha, Sankhlaburi District, Kanchanaburi Province, Thailand. The study was approved by the Ethical Review Committee of the Thai Ministry of Public Health and the U.S. Army Human Subjects Research Review Board. Informed consent was provided by all adults (20 years of age and older) participating in the study and by a parent or legal guardian of children 1–19 years old.

The Department of Entomology has maintained a field laboratory in Ban Kong Mong Tha since June 1999. This isolated village is located within a narrow riverine valley that

has an area of approximately 4 km². The village is located at an elevation of 180 m above sea level, and is surrounded by mountains with a maximum height of approximately 900 m. The village itself consists of approximately 650 individuals living in 120 houses. Malaria occurs throughout the year in Ban Kong Mong Tha, with an average monthly prevalence of approximately 10%. A total of 7,801 blood films were collected between May 2000 and October, 2001, with 313 (4.0%), 407 (4.9%), and 9 (0.1%) blood films containing *P. falciparum*, *P. vivax*, and *P. malariae*, respectively. The majority of malaria cases are asymptomatic. A total of 9,618 adult anopheline mosquitoes were collected between June 1999 and September 2001. Mosquitoes were collected as they attempted to bite human volunteers between 6:00 PM and 6:00 AM the following day. The predominant anophelines collected were *Anopheles minimus* (54%), *A. sawadwongporni* (14%), *A. maculatus* (13%), *A. campestris* (5%), and *A. barbirostris* (4%), with specimens of the first four species testing positive for circumsporozoite protein by an enzyme-linked immunosorbent assay. Data on the epidemiology of malaria in Ban Kong Mong Tha will be reported separately.

Patients. A total of 585 individuals living in the village ($N = 651$) chose to enroll in the study. Each month, three separate teams went house to house and made blood films from all consenting individuals who were available during a three-day (Monday–Wednesday) period. At the time of fingerprick, each individual was questioned about illness (fever, headache, muscle aches, and pains), travel history (travel that required an overnight stay outside the limits of the village), and medications taken during the previous two weeks.

Microscopy. Thick and thin films were prepared directly from fingerprick blood samples. Thick and thin films were stained with 10% Giemsa solution and examined at the field site by three trained microscopists from the Sankhlaburi Dis-

TABLE 1

Plasmodium falciparum (Pf) and *P. vivax* (Pv) malaria in individuals from the village of Ban Kong Mong Tha participating in the study

Age (years)	Female			Male		
	Individuals sampled	Total blood films prepared	Total films positive (Pf/Pv)	Individuals sampled	Total blood films prepared	Total films positive (Pf/Pv)
1-4	34	187	16 (8.6%)*	49	266	19 (7.1%)*
5-9	40	235	27 (11.5%)*	47	290	38 (13.1%)
10-14	44	230	25 (10.9%)*	52	281	57 (20.3%)
15-19	11	55	6 (10.9%)	30	121	10 (8.3%)
20-29	45	241	21 (8.7%)	33	136	26 (19.1%)
30-39	41	242	19 (7.9%)	47	200	14 (7.0%)
40-49	23	125	13 (10.4%)	27	141	17 (12.1%)
>50	27	118	11 (9.3%)	34	136	18 (13.2%)
Total	265	1433	138 (9.6%)	320	1571	199 (13.4%)
Total individuals = 585			Total blood films = 3,004		Total positive films = 337	

* Includes one mixed Pf/Pv infection (total = 4).

strict malaria clinic who were participating in the study (field microscopy). Films were examined at $\times 700$ using natural light. The initial thick film was considered negative if no parasites were seen in at least 50-100 fields. Each film required approximately five minutes to read. For positive slides, species and presence or absence of gametocytes was recorded. The microscopist was unaware of any clinical diagnosis at the time each blood film was read.

All blood films were subsequently examined at $\times 1,000$ by an expert microscopist (N.M.) with more than 25 years of experience. The expert microscopist was blinded to the clinical diagnosis and the initial field microscopy result. The parasite density was counted per 500 leukocytes and was then expressed as the number of trophozoites and gametocytes per microliter by assuming a leukocyte count of 7,000/ μL . The initial thick film was considered negative if no parasites were seen in at least 100 high-power fields (more if fewer than 500 leukocytes were observed per 100 fields). A high-quality (Olympus, Melville, NY) microscope with an incandescent light source was used. Each film required approximately 20 minutes to read.

All slides with discordant results and 20% of slides with concordant results were cross-checked in Bangkok by an expert microscopist with over 20 years experience. The microscopist was also blinded to the clinical diagnosis and to previous microscopy results.

Treatment. The name, age, and house number of all individuals with a malaria-positive blood film were provided to Thai Ministry of Public Health (MOPH) workers participating in the study. All malaria-positive individuals were treated according to the malaria treatment protocols of the Thai MOPH. Treatment follow-up was provided by Thai MOPH workers participating in our study and in accordance with Thai MOPH standard procedures.

Data analysis. Epi-Info (version 6; Centers for Disease Control and Prevention, Atlanta, GA)⁶ was used to calculate field microscopy performance and acceptability evaluation indices, with expert laboratory microscopy used as the gold standard. Performance indices were calculated for each of the following microscopic diagnoses: malaria as a whole (diagnosis of either *P. falciparum* or *P. vivax*), *P. falciparum* malaria (including mixed infection), and *P. vivax* malaria. The variables measured were the number of true positive (TP), true negative (TN), false positive (FP), and false negative (FN) results. Sensitivity was calculated as $\text{TP}/(\text{TP} + \text{FN})$, specificity was calculated as $\text{TN}/(\text{TN} + \text{FP})$, the positive predictive value

(PPV) was calculated as $\text{TP}/(\text{TP} + \text{FP})$, and the negative predictive value (NPV) was calculated as $\text{TN}/(\text{FN} + \text{TN})$. Sensitivity and specificity were used to calculate the likelihood ratios for a positive test result [$\text{sensitivity}/(1 - \text{specificity})$] and a negative test result [$(1 - \text{sensitivity})/\text{specificity}$].⁷ The likelihood ratios were used to determine post-test probabilities by using the nomogram of Fagan.⁸ Test accuracy, the proportion of all tests that gave a correct result, was defined as $(\text{TP} + \text{TN})/\text{number of all tests}$. Reliability was expressed as the J index $(\text{TP} \times \text{TN} - \text{FP} \times \text{FN})/(\text{TP} + \text{FN})(\text{TN} + \text{FP})$.⁹

In the analysis for malaria as a whole, incorrect determination of the species was considered a false-positive result because treatment will vary based on accurate species identification. Because treatment of *P. falciparum* infections will eliminate *P. vivax* trophozoites but the converse is not necessarily so, when analyzing performance of field microscopy, mixed infections detected by expert laboratory microscopy were considered true negatives if field microscopy only detected *P. vivax* and true positives if field microscopy detected only *P. falciparum* or both *P. vivax* and *P. falciparum*.

RESULTS

Of the 585 individuals who enrolled in the study, 320 (54.7%) were males and 265 (45.3%) were females. The age range was 1 to 90, with 266 (45.5%) less than 15 years of age (Table 1). A total of 3,004 blood films were made from these 585 individuals, of which 156 (5.2%) were positive for *P. falciparum*, 177 (5.9%) for *P. vivax*, 4 (0.1%) for both *P. falciparum* and *P. vivax*, and 2,667 (88.8%) were negative for both *P. vivax* and *P. falciparum* by expert microscopy (Table 2). In

TABLE 2

Comparison of field and expert laboratory microscopy for active surveillance for *Plasmodium falciparum* and *Plasmodium vivax*

Expert microscopy result	No. of samples with the following result by field microscopy				Total
	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. falciparum</i> and <i>P. vivax</i>	Negative	
<i>P. falciparum</i>	14	5	0	137	156
<i>P. vivax</i>	3	12	0	162	177
<i>P. falciparum</i> and <i>P. vivax</i>	1	0	1	2	4
Negative	18	14	0	2,635	2,667
Total	36	31	1	2,936	3,004

TABLE 3

Performance characteristics of field microscopy relative to those of expert laboratory microscopy for active surveillance for *Plasmodium falciparum* and *Plasmodium vivax**

Expert microscopy result	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])	Accuracy (%)	J index	Likelihood ratio	
							Positive test result	Negative test result
Total	8.3% (5.7–11.9)	98.8% (98.3–99.2)	46.7% (33.9–59.9)	89.5% (88.3–90.6)	88.6%	0.07	6.9	0.93
<i>P. falciparum</i>	10.0% (6.0–16.0)	99.3% (98.9–99.5)	43.2% (27.5–60.4)	95.1% (94.3–95.9)	94.5%	0.09	14.3	0.91
<i>P. vivax</i>	7.1% (3.9–12.4)	99.2% (98.8–99.5)	38.7% (22.4–57.7)	93.9% (92.9–94.8)	93.8%	0.07	10.3	0.93

* CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

addition, two films (taken one month apart from the same individual) were positive for *P. malariae*.

Nine percent (14 of 156) of *P. falciparum*-infected individuals, 5.7% (10 of 176) of *P. vivax*-infected individuals, 50% (2 of 4) of individuals infected with both *P. falciparum* and *P. vivax*, and 5.4% (143 of 2,667) of uninfected individuals were febrile at the time the blood film was made. Other symptoms (headache, muscle ache, respiratory difficulty, etc.) were reported in only 1.3% (2 of 156) of *P. falciparum*-infected individuals and in none of the other individuals.

The results of parasite detection by expert laboratory microscopy and field microscopy are shown in Table 2. Field microscopy was specific (99.3%) but not sensitive (10.0%) for the diagnosis of *P. falciparum* malaria, with a PPV of 43.2% and an NPV of 95.1% (Table 3). The corresponding sensitivity and specificity for the diagnosis of *P. vivax* malaria were 7.1% and 99.2%, respectively, with a PPV of 38.7% and an NPV of 93.9%. Other evaluation indices are shown in Table 3. Test sensitivity for the detection of both *P. falciparum* and *P. vivax* increased with increasing parasitemia (Table 4): sensitivity of field microscopy for the detection of both *P. falciparum* and *P. vivax* was 100% for each with parasitemias $\geq 5,000/\mu\text{L}$, 16.7% and 33.3% for *P. falciparum* and *P. vivax*, respectively, with parasitemias between 500 and 4,999/ μL , and only 5.1% and 7.1% when parasitemias were between 50 and 500/ μL . Sensitivity for both *P. vivax* and *P. falciparum* was 0% with parasitemias less than 50/ μL .

DISCUSSION

A variety of studies have clearly demonstrated that microscopic diagnosis of malaria can vary greatly in its accuracy, particularly at low parasitemia rates.^{2–4} This variation in specificity and sensitivity is routinely observed in clinical settings, where a high percentage of reporting patients are parasitemic and parasite densities are relatively high. It was therefore not surprising that field microscopy was not as accurate as expert laboratory microscopy when used for active malaria surveillance in Thailand, where prevalence rates (11.2% overall, 337 of 3,004) and parasite densities (mean density = 52.6/ μL for *P. falciparum* and 24.2 for *P. vivax*) are low. Although specificity of field microscopy was acceptable (99.3% and 99.2% for *P. falciparum* and *P. vivax*, respectively) when compared with expert laboratory microscopy, the low sensitivity (10.0% and 7.1% for *P. falciparum* and *P. vivax*, respectively) was not.

In a recent review of practical techniques for the diagnosis of malaria,¹ Makler and others concluded that there is no single, standard method used by all investigators for the quan-

tification of parasites in thick smears. They concluded that a variety of factors, which included under-trained microscopists, lack of microscopes and staining material, and processing and reading large numbers of blood smears, dramatically increased the room for error.

Results from this study clearly demonstrate that variation in microscopic detection can have an even more marked impact during active surveillance for malaria, where the overall prevalence and parasitemia rates are far lower than normally encountered during passive surveillance (typically evaluation of symptomatic patients reporting to a local malaria clinic). For example, Barat and others¹⁰ found that an average of 29% of persons referred for blood slide examination at health centers in Zambia had a positive reading for malaria parasites, while Hemme and Gay⁵ found that prevalence rates in Thailand ranged from 49% to 77% during passive surveillance. More than 85% of children (697 of 815) and 54.8% of adults with presumptive *P. falciparum* infections reporting to health centers in Papua New Guinea had parasite rates $> 1,000/\mu\text{L}$.¹¹ Similarly, in eastern Indonesia, 78% of symptomatic test subjects infected with *P. vivax* had parasite rates greater than 500/ μL .¹ In contrast, only 11.2% (337 of 3,004) of films examined in this study were positive, with only 15.6% (25 of 160) and 6.1% (11 of 180) of *P. falciparum*- and *P. vivax*-positive slides, respectively, having parasite rates of $\geq 500/\mu\text{L}$.

The data obtained in this study furthermore suggest that under the conditions described (low parasite prevalence, low parasite rates, and inadequate equipment), conducting active malaria surveillance using field microscopy is not justified. The low sensitivity of field microscopy indicates that the technique will only identify individuals with relatively high parasite levels. These are the very individuals who would normally wind up seeking treatment at a local malaria clinic in Thailand. In fact, three of seven individuals with *P. falciparum* levels greater than 5,000/ μL and two of two individuals with

TABLE 4

Performance of field microscopy relative to expert laboratory microscopy at different *Plasmodium falciparum* and *Plasmodium vivax* trophozoite densities

Trophozoite/ μL	<i>P. falciparum</i>		<i>P. vivax</i>	
	No. positive by expert microscopy	No. correct by field microscopy	No. positive by expert microscopy	No. correct by field microscopy
<50	18	0 (0%)	57	0 (0%)
50–499	117	6 (5.1%)	113	8 (7.1%)
500–4,999	18	3 (16.7%)	9	3 (33.3%)
>5,000	7	7 (100%)	2	2 (100%)
Total	160	16 (10.0%)	181	13 (7.2%)

P. vivax parasites levels greater than 5,000/ μ L were symptomatic at the time the blood film was taken, and probably would have reported to the local malaria clinic seeking treatment.

The goal of active surveillance is presumably to identify those individuals who would normally not be detected in a passive (clinic based) surveillance program, i.e., asymptomatic individuals. It is these very individuals who normally have low parasite rates, thereby making parasite detection that much more difficult. If active surveillance is to be undertaken, it should only be conducted using high-quality equipment (to include an artificial light source) combined with rigorous quality control standards for slide examination. In areas such as western Thailand where prevalence and parasite levels are low, active surveillance is a highly labor intensive effort that will provide minimal returns.

Acknowledgments: This study would not have been possible without the willing participation of the residents of Ban Kong Mong Tha and the staff of the Sangklaburi Malaria Clinic. We are grateful to N. Chuanak and B. Permpnich of the Department of Immunology and Medicine, Armed Forces Research Institute of Medical Sciences, for their assistance as secondary expert microscopists.

Financial support: Funding for this project was provided by the Military Infectious Diseases Research Program of the U.S. Army Medical Research and Materiel Command (Fort Detrick, Frederick, MD).

Disclaimer: The opinions or assertions contained in this manuscript are the private ones of the authors and are not to be construed as the official or reflecting views of the Department of Defense or the Armed Forces Research Institute of Medical Sciences.

Authors' addresses: Russell E. Coleman, Nongnuj Maneechai, Nat-tawan Rachaphaew, Chalernpol Kumpitak, R. Scott Miller, and Jetsumon Sattabongkot, United States Army Medical Component, Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok, 10400 Thailand. Virat Soyseng, Sankhlaburi District Malaria Clinic, Kanchanaburi Province, Thailand. Krongthong Thimasarn, Malaria Division, Department of Communicable Disease Control, Nonthaburi, Thailand.

Reprint requests: Department of Entomology, Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand.

REFERENCES

1. Makler MT, Palmer CJ, Ager AL, 1998. A review of practical techniques for the diagnosis of malaria. *Annals Trop Med Parasitol* 92: 419-433.
2. Warhurst DC, Williams JE, 1996. Laboratory diagnosis of malaria. *J Clin Pathol* 49: 533-538.
3. Craig MH, Sharp BL, 1997. Comparative evaluation of four techniques for the diagnosis of *Plasmodium falciparum* infections. *Trans R Soc Trop Med Hyg* 91: 279-282.
4. Tham JM, Lee S, Tan TM, Ting RC, Kara UA, 1999. Detection and species determination of malaria parasites by PCR: comparison with microscopy and with ParaSight-F and ICT malaria Pf tests in a clinical environment. *J Clin Microbiol* 37: 1269-1273.
5. Hemme F, Gay F, 1998. Internal quality control of the malaria microscopy diagnosis for 10 laboratories on the Thai-Myanmar border. *Southeast Asian J Trop Med Public Health* 29: 529-536.
6. Dean AG, Dean JA, Coulombier D, Brendel KA, Smith DC, Burton AH, Dicker RC, Sullivan K, Fagan RF, Arner TG, 1995. *Epi Info, Version 6: A Word-Processing Database, and Statistics Program for Public Health on IBM-Compatible Microcomputers*. Atlanta, GA: Centers for Disease Control and Prevention.
7. Jaeschke R, Guyatt G, Sackett DL, 1994. Users' guide to the medical literature. III. How to use an article about a diagnostic test, A. Are the results of the study valid? *JAMA* 271: 389-391.
8. Fagan T, 1975. Nomogram for Bayes' theorem. *N Eng J Med* 293: 257.
9. Mharakurwa S, Manyame B, Shiff CJ, 1997. Trial of the ParaSight-F test for malaria diagnosis in the primary health care system, Zimbabwe. *Trop Med Int Health* 2: 54-55.
10. Barat L, Cjipipa J, Kolczak M, Sukwa T, 1999. Does the availability of blood slide microscopy for malaria at health centers improve the management of persons with fever in Zambia? *Am J Trop Med Hyg* 60: 1024-1030.
11. Genton B, Smith T, Baea K, Narara A, Al-Yaman F, Beck H-P, Hii J, Alpers M, 1994. Malaria: how useful are clinical criteria for improving the diagnosis in a highly endemic area? *Trans R Soc Trop Med Hyg* 88: 537-541.