FIELD APPLICATION AND EVALUATION OF A RAPID IMMUNOCHROMATOGRAPHIC TEST FOR DETECTION OF *PLASMODIUM FALCIPARUM* INFECTION AMONG THE INHABITANTS OF LAO PDR

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Abstract. Field application and evaluation of a rapid immunochromatographic test (ICT) for detection of *Plasmodium falciparum* infection were performed in 13 villages in a southern province of Lao PDR in 1999. More than 2,000 inhabitants, accounting for 61.8% of the total estimated population, were examined. Malaria infection was confirmed in all villages surveyed by ICT and microscopic diagnosis. The positive rates of *P. falciparum* malaria by microscopy ranged from 9.7% to 59.2% (mean 27.2%), whereas by ICT they were from 11.6% to 64.5% (mean 29.8%). The positive rates by ICT were generally higher in 8 out of 13 villages. However, a significant difference between the positive rates by microscopy and ICT was not observed in all villages. *Plasmodium falciparum* infection was actually confirmed by microscopy in 84.1% of specimens that tested positive by ICT. The results by ICT were consistent with those of the microscopic diagnosis, the discrepancy of the results was less than 10% (141/2,066). The ICT was falsely-positive in 4.7% and falsely-negative in 2.1% of the test cases. These results showed the efficacy of ICT not only in the diagnosis of the respective cases, but also in the mass-examination in the field.

INTRODUCTION

Lao PDR, a landlocked Southeast Asian country, is an endemic area for malaria (Giboda *et al*, 1992; Pholsena, 1992). In our recent active surveys based on microscopy of Giemsa-stained thick and thin blood films, positive rates of malaria from 2% to more than 50% have been demonstrated among the inhabitants in many villages in a southern province (Kobayashi *et al*, 1998, 2000; Toma *et al*, 2001; Kobayashi, unpublished data). The current malaria situation in Lao PDR is a serious public health problem not only in this country, but also in bordering countries. The operation of an effective malaria control program, is being developed for Lao PDR. One of the more effective control measures of malaria may be active mass-examination and subsequent mass-treatment of the inhabitants. The difficulty for such a mass-control program in this country seems to be that about 85% of people are living in remote rural areas where the inhabitants have little access to any health care system, especially in the rainy season. Many of the remote areas become inaccessible both by transportation and communication procedures during heavy rains.

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Although microscopic diagnosis of blood samples is the only standard method to detect malaria infection in the country, there are inherent problems with this method: lack of skilled microscopists, limited supply of equipment, inadequate quality control, and the time element. The delay in the results of microscopic diagnosis is a serious obstacle for the operation of a mass control program in inaccessible remote areas where health sector personnel operating the control program have to visit several times to treat the positive subjects, as per the results of microscopic examinations.

In the present study, the authors tried to apply a rapid immunochromatographic test, for an active mass survey to detect *Plasmodium falciparum* infection among inhabitants in Lao PDR which can be performed within 5 minutes and allow simultaneous treatment in a single survey operation.

MATERIALS AND METHODS

Study areas and subjects examined

The studies were performed in December (dry season), 1999 in 13 villages in Boualapha district, Khammouane Province. The surveys were conducted under the joint collaboration with the study team of the Center of Malariology, Parasitology and Entomology (CMPE), Vientiane, Lao PDR.

The total estimated population in the villages was 3,345 and a total of 2,066 (61.8%) were actually examined. Among the subjects, children under 15 years old accounted for more than 50% of the subjects. The number of female subjects was 1.3 times that of males. All villagers testing positive following an immunochromatographic test (ICT) were promptly treated with chloroquine or Fansidar®.

Collection of blood

From a single finger-prick, thick and thin blood smears for microscopy were prepared on the same glass slide and stained with 10% Giemsa solution. Approximately 10 μl of blood for immunochromatography testing was also drawn into an EDTA-treated capillary tube provided with the test kits and the capillary tube blood was promptly transferred onto the sample pad of the test kit.

Immunochromatographic testing

The ICT Malaria Pf Test™ (AMRAD-ICT, Sydney, Australia) for detection of *P. falciparum*-specific HRP2 antigen was used in the present survey (Parra *et al*, 1991; Beadle *et al*, 1994). The test was performed by well-trained workers. After spotting approximately 10 μl of whole blood onto a sample pad, buffer drops (Reagent A) were added per manufacturer's instructions. A test line and positive control line were allowed to develop for 5 minutes and the results were read by one observer to avoid inter-observer variation. For each test, the test line intensity was compared to the internal control line and graded using a (-) to (+) scale as follows: (-), no test line, negative; (±), positive test line, but very faint; (+), positive test line.

Microscopic diagonsis

Geimsa staining and slide interpretation was done by experienced microscopists from the CMPE according to standard operating procedure. The initial thick film was considered negative if no parasite was seen in at least 100 high-power fields. If malaria parasites were seen, the species was identified based on its morphological features on the associated thin smear.

Data analysis

The variables measured were the number of positives both by microscopy and the ICT (PB), of negatives both by microscopy and ICT (NB), of positives only by ICT (PI), and of negatives only by ICT (NI). The sensitivity was calculated as PB/(PB+NI) and specificity was calculated as NB/(NB+PI).

Statistical analysis was performed by either χ^2 test or the linear regression analysis using the computer statistical software program (SPSS). The probability (p value) less than 0.05 was considered to be statistically significant.

RESULTS

The results obtained are summarized in Table 1. Malaria was confirmed in all villages surveyed. The positive rates of *P. falciparum* malaria by microscopic examination ranged from 9.7% to 59.2% and the mean rate was 27.2%. On the other hand, the positive rates by the ICT were from 11.6% to 64.5%, showing a mean rate of 29.8%. The positive rates by the ICT were generally higher in 8 out of 13 villages but a significant difference between positive rates by microscopy and ICT was observed only in one village (Koutboun). The sensitivity and specificity of ICT were also represented in Table 1 by each village. The sensitivity ranged from 82.8% to 100% and the specificity was from 72.4% to 100% in these villages.

The relationship between the demonstration of malaria parasites by microscopy and the positivity in ICT is summarized in Table 2. As

Table 1
Results of ICT and microscopic examination for *Plasmodium falciparum* infection among the villagers in 13 villages in dry season (December, 1999).

District, village	No. examined[a]	No. positive (%)		Sensitivity (%)	Specificity (%)
		Microscopy	ICT		
Xebangfay district					
Sorn	271	43 (15.9)	43 (15.9)	90.7	98.2
Khoktong	257	47 (18.3)	47 (18.3)	89.4	97.6
Thamlay	319	31 (9.7)	37 (11.6)	96.8	97.6
Boualapha district					
Thahe	105	50 (47.6)	60 (57.1)	100	81.8
Paknay Neua	68	33 (48.5)	32 (47.1)	97.0	100
Napoung	256	61 (23.8)	67 (26.2)	88.3	92.7
Paknay Tai	76	45 (59.2)	49 (64.5)	93.3	77.4
Nakachan Tha	111	50 (45.0)	53 (47.7)	90.0	86.9
Koutboun	163	76 (46.6)	95 (58.3)[b]	93.4	72.4
Nalouang	91	52 (57.1)	51 (56.0)	92.3	92.3
Khok	138	27 (19.6)	35 (25.4)	100	92.8
Tapachone	121	29 (24.0)	27 (22.3)	82.8	96.7
Pungbone	90	17 (18.8)	20 (22.2)	88.2	93.2
Total	2,066	561 (27.2)	616 (29.8)	92.2	93.5

[a]Number of specimens examined both by microscopy and ICT.
[b]The positive rate was statistically different from that by microscopy.

Table 2
Comparison of results by microscopic examination and ICT for detection of *Plasmodium falciparum* infection among the inhabitants of 13 villages in Kammouane Province, Lao PDR.

Microscopic examination	ICT[a]		Total (%)
	Positive (%)	Negative(%)	
Positive	518 (25.1)	43 (2.1)	561 (27.2)
Negative	98 (4.7)	1,407 (68.1)	1,505 (72.8)
Total	616 (29.8)	1,450 (70.2)	2,066 (100)

[a]The sensitivity of the ICT calculated was 92.3% with the specificity of 93.5%.

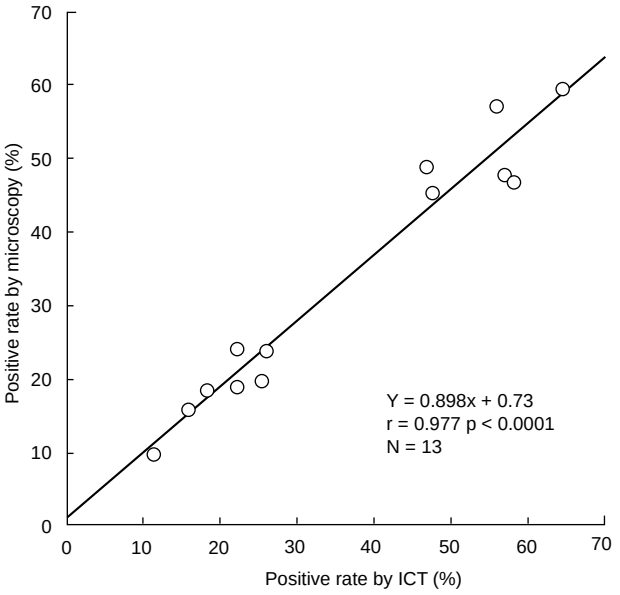

Fig 1–A scatter diagram of positive rate of *Plasmodium falciparum* infection by microscopic examination and ICT and correlation between the two tests performed in 13 villages.

a total, *P. falciparum* infection was actually confirmed by microscopy in 84.1% of specimens tested positive by ICT. On the other hand, 43 specimens, accounting for 2.1% of total specimens, did not show positive results by ICT, in spite of the demonstration of *P. falciparum* parasites by microscopic examination. The specimens which were positive by the ICT but not by microscopy occupied 4.7% of the specimens examined. The overall specificity and sensitivity of the ICT calculated according to the results was 92.2% and 93.5%, respectively.

Fig 1 represents the correlation between positive rates by the ICT and microscopy. The positive rate by the ICT was significantly correlated with that by microscopy ($r=0.977$, $p<0.0001$).

DISCUSSION

Lao PDR covers an area of approximately 240,000 km^2 in Southeast Asia. The estimated population by census in 1996 was 5.0 million. A large part of the country is mountainous and inaccessible. The climate is tropical, the rainy season is from May to October. In this country, malaria is known to be seriously endemic among the inhabitants (Pholsena, 1992). Although almost all of the data on the current prevalence of malaria in Lao PDR was based on the number of passively detected cases; the official record of malaria cases in 1998 indicated the occurrence of 307,189 cases, including 588 fatal cases. Khammouane Province surveyed in the present study is a southern province which covers about 8% of the country's surface with an estimated population of 170,000 in 1995. The province is also known as a highly endemic area for malaria; a total of 1,630 cases including 24 fatalities were reported in the same year.

On the other hand, there have been few active surveys on the inhabitants to estimate pre-patent or latent infection in this country. In a past survey in the northern areas (Keodom District, Vientiane Province), in which a total of 1,105 villagers accounting for 7.0% of the total population subjected were actively examined for malaria infection, the positive rate was reported to be 2.4% among the villagers (Giboda *et al*, 1992). Most recently, the author and his co-workers have indicated that malaria is more seriously prevalent among the inhabitants in many areas in the southern Khammouane Province, showing that the mean positive rate by microscopy was 16.4% in 26 villages (Kobayashi, unpublished data).

These prevalence rates determined by microscopy, however, may sometimes underestimate true prevalence because of many latent infections among the inhabitants (Elhassan *et al*,1995; Farnert *et al*, 1997; Babiker *et al*, 1998). The authors have also demonstrated in recent active surveys that, if blood samples were examined simultaneously by polymerase chain reaction assay, the prevalence rate was 2-times higher than that by microscopy (Toma *et al*, 2001). In the present study, therefore, a rapid ICT test for the detection of *P. falciparum*

infection, which accounts for more than 85% of malaria detected in the country, was further applied to evaluate field efficacy for the mass screening survey. The positive rate by ICT was 29.8% which was slightly higher than that by microscopy. The results, however, were consistent with those by microscopy, showing the discrepancy of results between ICT and microscopical examination was less than 10% (141/2,066). The ICT was falsely-positive in 4.7% and falsely-negative in 2.1%. These results confirmed the efficacy of ICT not only in diagnosis of individual cases but also in the mass screening in the field.

The use of ICT for mass diagnosis seems to be applicable for a malaria control program, because of its simple procedure and only 5 minutes of performing time. By application of ICT, malaria positive inhabitants could be treated readily and on the same occasion of mass diagnosis in the present study. The most important point for the villagers is knowledge that they are infected with malaria parasite, as well as dispersing any information for preventive measures of the disease. The mass diagnosis using the ICT, therefore, would be effective enough to attract the villager's attention and persuade them to participate in a control program.

The difficulty in applying the ICT for such a mass screening survey in many villages seems to be the high unit price of testing kits as compared with the ordinary method, Giemsa staining. The total cost of mass control program using the test including transportation fees and personnel expenses is, however, not always higher in comparison with that using the Giemsa staining method, in which several return trips to the same village are necessary to treat malaria-positive persons. It is especially true in Lao PDR where half the villages are located in remote areas and are hard to access from central districts by using conventional transportation methods. The ICT seems to have a great value for a malaria control program in the Southeast Asian region where highly endemic areas of malaria consist mainly of remote villages, unlike Africa.

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