INTERNAL QUALITY CONTROL OF THE MALARIA MICROSCOPY DIAGNOSIS FOR 10 LABORATORIES ON THE THAI - MYANMAR BORDER

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Abstract. On the Thai-Myanmar border, where multidrug resistance to anti-malaria medications is a major problem, a quality control program for diagnostic laboratories has been set up. This study examines the "passive" screening performed in 10 laboratories. Monthly evaluation of the quality of thick and thin smear practice, Giemsa staining and microscopy took place during the year 1994. Considering the general context and the methodology applied, the evaluation of performance and strategy of the malaria diagnostic test showed satisfactory results for all 10 laboratories. Performance of technics = 64% (62-66) to 96% (95-97); Sensitivity = 92.6 (91.5-95.5) to 96.6% (95.8-99.0); Specificity = 93.5% (91.4-95.5) to 98.3% (97.6-99.0); Predictive Positive Value = 92.0% (90.9-93.1) to 98.3% (97.6-99.0); Predictive Negative Value = 94.3% (93.0-95.6) to 98.5% (98.0-99.0). The study underlines the importance of a reliable quality control method for microscopy diagnosis of malaria in hyperendemic areas, with *Plasmodium falciparum* as the main species. A high level of input from the international laboratory technician, performing training, follow-up and evaluation was required. The need for adequate training of national technicians and supervisors, especially regarding long-term sustainability, is stressed. The type of program presented can be used as a model for similar projects in developing countries.

INTRODUCTION

Without the possibility of laboratory diagnosis of malaria, clinicians in the field in contexts such as on the Thai-Myanmar border, have to rely on solely clinical diagnosis. This will lead not only to an excess of patients treated for malaria, especially when presenting with hyperthermia, but worse to delayed or no treatment of patients being affected. Diagnosis performed through microscopy in a laboratory, its quantitative and qualitative parasitological parameters, play a major part in improving not only the diagnosis, but also the treatment and follow-up of malaria patients.

As stipulated in the eighteenth report of the WHO committee of malaria experts: "... of all the anti-malaria activities, the two most important are to diagnose in time and to treat the patient". (WHO, 1986)

In view of the rapidly increasing multidrug resistance of the malaria parasites along the Thai-Myanmar border and the complexity of the treatment protocols, the set-up of laboratories, as part of the malaria control program, is a priority. The use of microscopy for the diagnosis allowed the differentiation of the species, and therefore the choice of medication required, as well as the reduction of false negatives essential in view of the potentially lethal *Plasmodium falciparum* infections.

Indeed the identification of the parasite in the blood as a diagnostic test has to be made available as soon as possible, within malaria control programs. It allows not only confirmation of the clinical diagnosis and identification of the species, but also the determination of the parasitemia. The results enable the health staff to choose and follow the required treatment protocols, the parasitemia being relevant especially for the surveillance of the progress of patients with a positive malaria diagnosis.

Efficiency of laboratories, and the reliability of results such as the diagnosis of malaria through the use of microscopy, set up as part of the primary health care structures in developing countries will depend on a variety of factors such as the competence of the laboratory technicians, regular practice in the use of the tests with sufficient experience in the diagnosis even in samples with low parasitemia,

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the quality of the equipment and the regularity of the supplies, and adequate organization of the structures and management of the staff (Payne, 1988).

The main objective of this study has been to establish a quality control program for the laboratory diagnosis in health structures located on both sides of the Thai-Myanmar border during the year 1994. Specific objectives are to evaluate the quality of the performance of thin and thick blood smears, the quality of the Giemsa staining and the quality of the microscopy reading.

MATERIALS AND METHODS

Patients

The "passive" screening (*ie* patients reporting to the health units, seeking treatment in view of existing signs and symptoms) and quality control program has been established over an area of ~ 400 km, covering both sides of the Thai-Myanmar border. It affected three different types of populations:

- A population of refugees and internally displaced persons, belonging to the Karen and Mon ethnic minorities, who were at the time of this study, living in camps at the northern (Mae Sot area, mostly Karen people) and southern parts (Sangkla Buri area, mostly Mon people) of the Thai-Myanmar border. The size of these populations has been estimated at around 9,500 persons in the north (census performed by MSF in July 1994) and 6,400 persons in the south (census performed by MSF in June 1994).

- A population of internally displaced, mostly Karen people, living in villages in the north eastern part of Myanmar (Karen state, Duplaya district) in relative insecurity. This population, having access to the district health structures, was estimated by the district health chairman to around 19,000 persons for the year 1994.

- A mixed population, consisting of internally displaced persons and villagers from Myanmar, crossing the border for consultations and treatment at a health clinic "Dr Cynthia's clinic", located at Mae Sot in Thailand. According to the clinic's register the number of patients during the year 1994, had been around 5,000.

Participated laboratories

Ten health structures, each with a laboratory, have been chosen for this study:

- For the Karen people: the health units of the two camps of Wangka (WK) and Mawker (MK)

- For the Mon people: the health units of the two camps of Payaw (PY) and Halokhani (HLK)

- For the internally displaced and villagers in Myanmar: the health units of Sakhan Htit (SKT), Paw Daw Moo (PDM), Kyaik Don (KD), Winlo (WL) and Quangalay (QGL).

- For the mixed population of internally displaced and Myanmar villagers, crossing the border to Thailand: "Dr Cynthia's clinic" (CYN).

The laboratories at SKT, WL and QGL were set up and opened in July and August 1994 and the others laboratories were set up before July 1994.

Malaria diagnosis

The diagnosis of malaria was systematically made through microscopic examination of a thick and a thin smear of a capillary blood sample, taken from the finger of the respective patient and stained for twenty minutes with 10% Giemsa solution.

With the exception of WL and QGL, equipped with solar monocular microscopes, and CYN, equipped with an electric binocular microscope, all laboratories were equipped with solar binocular microscopes. All microscopes were fitted with 40x and 100x oil-immersion objectives. To ensure adequate functioning and maintenance, the microscopes were stored in hermetic boxes, containing silicagel, during the hours of closure of the laboratories. Once a year the microscopes were sent to Bangkok for cleaning and if necessary for replacement of spare parts and repairs. An additional microscope allowed replacement during this period, so as not to interrupt the work in the respective laboratory.

The reading of the slides and the results were defined as follows: A negative slide was defined as absence of *Plasmodium* on both thin and thick smears, a positive slide as presence of *Plasmodium* on thin and/or thick smear. Parasitemia on positive slides was expressed in number of crosses, corresponding to a defined number of parasites per field, *ie* 1 to 2 parasites per field = (+), 3 to 25 parasites per field = (++), 26 to 60 parasites per field = (+++), over 60 parasites per field = (++++). The identification of the species and development stages was most often performed through examination of the

thick smear and subsequently confirmed through examination of the thin smear if in doubt.

All laboratory technicians evaluated received the same training of 4 to 5 weeks duration, including both theoretical and practical parts. In order to be admitted to the training, a number of selection criteria (*ie* school level, knowledge of basic calculation...) had to be fulfilled. At the end of the training, students had to sit for a final theoretical and practical examination and had to pass at least 80% of the tests successfully in order to qualify and be employed as laboratory technicians.

The teams working in the laboratories consisted of between two and five national laboratory technicians. Each team had one national supervisor, specifically trained for this post. Overall supervision of the national staff took place through regular field visits of one international laboratory technician based in Mae Sot. Despite problems of access during the rainy season and at times precarious security conditions, these supervisory visits have taken place every quarter, with a duration of one week for each visit, with the exception of CYN, MK and WK with monthly visits, lasting two to three days or more if necessary.

During the course of these visits the international technician performed the quality control examinations, spent time in supervision of both individual technicians and the team as a whole, trained the respective national supervisor for an increased involvement and responsibility regarding the supervision, discussed problems encountered and organized additional short training sessions as required. Teaching aids, such as a general supervision sheet and protocols for standard operating procedures, have been used for supervision and evaluation of the work.

Throughout the year 1994, monthly quality control examinations have been performed in all laboratories, with the exception of WK and MK, with quarterly controls in the beginning and monthly controls from September 1994 onwards. According to the workload and activities in the different laboratories, two protocols for the performance of the quality control have been established.

The first protocol was used for laboratories in SKT, PDM, KD, WL, QGL, HLK, PY and CYN. The national laboratory technicians kept all malaria slides collected during the month, classifying positive and negative slides separately. A random choice of 20% of all positive slides and 20% of all negative slides was performed. If the total number of slides collected during the respective month was below 120, a random choice of 50% of all positive slides and 50% of all negative slides was performed. The slides were then individually wrapped for protection during storage and, if necessary, transport.

The second protocol was applied in the laboratories in WK and MK. The national laboratory technicians kept all slides collected during the month in chronological order , regardless of whether the result had been found positive or negative. A random choice of 5 to 10% of all slides was performed and again the slides wrapped up individually for protection.

The evaluation of thick and thin smear technic as well as the Giemsa staining was performed with the protocols for standard operating procedures and through examination of the slides by the international laboratory technician. The national technicians' technic of reading the slides using the microscope was evaluated most of the times under field conditions, with slides done by the respective technician and being examined under the microscope available in the laboratory. After reading of the slide by the local technician, classifying the slide as positive or negative, the international laboratory technician repeated the reading, aware of the result issued by the national technician. Illegible slides have been discarded. All slides which were found either false positive or false negative, were reviewed jointly by the national and the international laboratory technician. All slides found positive by both the national and the international technician, but with a difference as to the species identified, were included as true positive slides in the contingency table.

The calculation of the proportion of slides taken, regardless of whether results were found positive or not, divided by the population is not presented with a confidence interval because of the "passive" screening being exhaustive. The denominators have been obtained either from data of a census performed in 1994 or collected from the respective health unit's register as total number of consultations. The calculation of the rate of the quality of the smear technic allows to appreciate the differences between the sites. The calculation of sensitivity and specificity for each laboratory, allowed us to assess the test as a diagnostic tool for malaria.

The predictive values, required in order to determine the performance of this strategy, have been calculated using the estimated prevalence for the respective region for the laboratories applying the first protocol, and the contingency table for the laboratories in MK and WK, applying the second protocol. The correlation between sensitivity and specificity has been calculated using the non-parametric correlation of Spearman's rank for all laboratories.

RESULTS

The number of blood films performed through "passive" screening, divided by the respective population, shown in Table 1, reflects the level of activity of the different sites. The latter varied with factors such as the number of staff, the capacity of the health unit, the access to the unit, especially during the rainy season, and, considering a variety of beliefs and cultural differences, its use by the population. Compared to the laboratories located inside Myanmar, the activity has been three times as important in the laboratory of "Dr Cynthia's clinic", and again twice as important than the latter in the camps in Thailand. The number of slides found positive, divided by the total number of slides, shown in Table 1, provides an estimate of the prevalence for each site, varying between 26% to 51% according to the origin of the population. The number of positive slides, divided by the population figures, shown in Table 1, provides an estimate of the incidence for the respective site. This estimate is considerably higher for sites in Thailand, *ie* 77%, 55% and 49%, than for sites in Myanmar *ie* 10%.

The monthly evaluation of the quality of the technic was set up in May 1994. The results are summarized in Table 2. Because of geographical reorganization of the work, the laboratories of MK and WK have been included from November 1994 onwards only. Amongst the laboratories located in Myanmar, SKT was opened only in July 1994, WL and QGL in August 1994. The reading technic was found to be better in the camps in Thailand when compared to "Dr Cynthia's clinic", $p < 10^{-5}$, results in the latter where again better than in the laboratories located in Myanmar, $p < 10^{-5}$.

Results for the measurement of the sensitivity, shown in Table 3, have been between 92.6% and 96.6%, for the specificity between 93.5% and 98.3%, varying at the different sites. The positive predictive value was found between 92.0% and 98.3%, the negative predictive value between 94.3% and 98.5%, again varying according to the different sites. The variation between laboratories has been more important for the positive predictive value than for the negative predictive value. The measurement of the non parametric correlation revealed a significant difference between sensitivity and specificity for the laboratory at HLK only ($r=0.75$ and $r^2=0.56$ with $p=0.005$).

DISCUSSION

Regarding the quantitative results, the variation of the level of activity within the different laboratiories varies with the location and population served. Some of the main factors influencing the activity are difficulties of access, especially during the rainy season, and the presence or absence of health education activities, which might contribute to patients consulting the health unit more frequently and at earlier stages.

For interpretation of prevalence figures it has to be kept in mind that some slides performed for the follow-up rather than the diagnosis might have been included, and could if excluded modify the figures obtained.

The difference between sites in the north and in the south, may be related to the fact that in the north, "Thai" and "Thai-Karen" populations, living nearby, but outside the camps were equally using the health structures, while health units in the south were used solely by the camp population.

Regarding the quality of the technic, the significant difference observed between camps has been very important. The differences are likely to be due to the fact that in some health units only the laboratory technicians performed the blood smears, whilst in others nurses and "medics" (locally trained national health staff) are participating by taking smears from patients. Despite training of other health staff by the international laboratory technician, for an improved performance of the smears, this factor has been difficult to control because of frequent changes and rotation of the health personnel outside the laboratory.

All technicians involved in this study have been

Table 1

Results of malaria slides performed in 1994 according to geographical areas.

	Northern Thailand		Myanmar	Southern Thailand
	MK and WK	CYN	SKT, PDM,KD QGL, WL	PY and HLK
Number of slides done	192%	107%	27%	188%
Population	n=9,500	n=5,000	n=19,000	n=6,400
Number of positive slides	40%	52%	36%	26%
Total number of slides	n=18,246	n=5,347	n=5,277	n=12,018
Number of positive slides	77%	55%	10%	49%
Population	n=9,500	n=5,000	n=19,000	n=6,400

n=size of the population estimated

Table 2

Evaluation of blood films and the staining during the year 1994.

	Northern Thailand		Myanmar	Southern Thailand
	MK and WK	CYN	SKT, PDM,KD QGL, WL	PY and HLK
Number of slides controled	555	814	1,975	1,813
Quality rate	95%	85%	64%	96%
IC 95%	(93-97)	(83-87)	(62-66)	(95-97)

IC = confidence interval

Table 3

Evaluation of performance of tests.

	Northern Thailand		Myanmar	Southern Thailand
	MK and WK	CYN	SKT, PDM,KD QGL, WL	PY and HLK
Se %	94.2	96.0	92.6	96.6
IC at 95%	(92.9-95.5)	(94.3-97.7)	(91.5-93.6)	(95.8-99.0)
Sp %	98.3	93.5	96.3	96.4
IC at 95%	(97.6-99.0)	(91.4-95.5)	(95.5-97.1)	(95.6-97.1)
PPV %	98.3	93.7	94.3	92.0
IC at 95%	(97.6-99.0)	(92.2-95.1)	(93.4-95.2)	(90.9-93.1)
PNV %	94.3	95.9	95.1	95.5
IC at 95%	(93.0-95.6)	(94.7-97.1)	(94.2-96.0)	(98.0-99.0)

Se = sensitivity, Sp = specificity, IC = confidence interval, PPV = positive predictive value, PPN = negative predictive value.

included in the quality control and have been supervised in the same way. A selection bias, which might have been introduced by performing the quality control only for certain technicians was therefore avoided. The quality control has been performed using slides taken and read by the respective laboratory technician and using the same microscope. It is difficult in this study to assess the impact of the technic for taking the blood smears on the reading of the slide as well as the quality of the equipment used.

The evaluation of the preparation of the slides, including both thin and thick smears, and the Giemsa staining, has been performed by one international laboratory technician, using standard operation procedure sheets as a written support. The use of these documents and the details of these evaluations decrease, but do not however succeed to exclude a certain degree of subjectivity on the side of the person performing the evaluation.

As indicated earlier on, national laboratory technicians have classified slides according to their results, *ie* positive or negative, prior to the performance of the quality control by the international technician. The internation technician was therefore aware of the results handed out by the national technicians before reading the slides. This may have lead to a performance bias with the consequence of a decreased difference in the results of both national and international technicians. Possible limitations and bias could be decreased through the use of an external evaluator and additional statistical advice should be sought regarding this issue (Goddard, 1980; Nawakowski, 1992).

In view of limitations due to the setting, context and method applied within this study, the measurement of performance and strategy of the malaria diagnosis by the laboratories reveal overall satisfactory results. Figures have been similar to previous studies performed in the north of Mae Sot between March 1992 and June 1993 (Lacroix, unpublished data). With the exception of CYN, the specificity has been better than the sensitivity. The positive predictive value between 92.0% and 98.3% and the negative predictive value between 94.3% and 98.5%, confirm the malaria diagnostic strategy as adequate. Regarding the one laboratory, HLK, where a significant difference between sensitivity and specificity has been found, no definite causative factor could be identified. For this laboratory 56% of the specificity can be explained by the sensitivity. The study stresses the importance of a reliable quality control of the laboratory diagnosis of malaria in hyperendemic areas with three species of *Plasmodium* (*ie* 85% to 80% of *Plasmodium falciparum,* 15% to 20% of *Plasmodium vivax,* 2% to 3% of *Plasmodium malariae*), the main species being *Plasmodium falciparum*. Areas of resistance are increasing and this type of program can be used as a model for similar projects in developing countries.

As the decision of malaria treatment may rely heavily on the laboratory examinations once they are available within a health structure, and therefore health staff will rely on the work performed by the laboratory technicians, the decision as to whether a laboratory should be opened for a health unit needs to be considered very carefully. Factors which will influence such a decision are likely to include: the size of the population served by the respective health unit, the number of patients treated, the availability of human resources, adequate training and supervision. Our experience during the course of this study would lead us to recommend the opening of a laboratory only if adequate quality and reliabilty of results can be ensured, as an important number of false results issued by the laboratory might have dramatic consequences for the patients and be indeed more harmful than for health staff to rely on their clinical diagnosis.

Regarding the qualitative results, following the training of new laboratory staff, certain factors observed during this study, have been found helpful: a close follow-up with frequent visits (*ie* at least twice a month) just after completion of the training, followed by quarterly visits, of a duration of one week per visit, three to six months later. Whenever the frequency of visits was decreased beyond this level, team dynamics and the quality of the work diminished, necessitating a longer supervisory visit.

The ease of maintenance of high reliability of the malaria diagnosis through the use of microscopy, in the context of developing countries, depends on a number of different factors. Qualification and experience of the trainer are of crucial importance, especially in the same person performs the evaluation of the national staff later on.

The necessity of a good interpreter needs to be stressed. The understanding of keys messages and main contents should continuously be checked.

Whenever possible the interpreter should be a laboratory technician with some practical working experience. During the course of this study, interpreters participating in training activities for laboratories in Myanmar and southern Thailand have been either laboratory technicians or others members of the health team, with, adequate knowledge of English, Karen or Mon and/or Myanmar language.

The level of activity within the respective laboratory is relevant, as for laboratories such as WL and QGL with a low total number of between 12 and 75 slides per month, the lack of practice may lead to poor technic and experience in reading of the slides. This factor should be considered even before the decision of the opening of a laboratory at the respective location is made.

The staff needs to be sufficiently competent and motivated; we wish to stress the importance of adequate selection criteria prior to the choice of candidates for training. A rotation of laboratory personnel within one region is possible and has taken place between laboratories in Duplaya district and Mon camps. We do not recommend a rotation of medical staff between duties such as curative posts and the laboratory, as performed in CYN, as amongst other difficulties, the quality of the work performed in the laboratory deteriorated. An additional factor observed as leading to poor functioning of the laboratory was the use of laboratory staff outside activities such as community work.

Each laboratory required the training of one national supervisor assisting with the enhancement and allowing an increased autonomy of the national team.

The equipment (*eg* microscopes, slides) and reagents (*eg* Giemsa, water pH7) need to be adequate in order to allow correct reading and to decreases the number of artefacts. Good organization will avoid problems such as registration errors, stock shortages and others.

Quality control in parasitology in the United States and in France is usually performed as an external evaluation. This has shown an improvement in the quality of the work performed, the evaluation looks mainly at the results issued by the laboratories (Nowakowski, 1992; Petithory *et al,* 1979). International organizations with projects in developing countries commonly perform internal evaluations, at time but not always followed by a second external evaluation. Internal quality control seems particularly important in the field of parasitology, as knowledge and practice of technicians are reflected in the performance of their work and do most often have an important and immediate impact on results issued (Smith, 1979; Leblanc, 1991). The internal quality control can be divided into five main areas:

- registration (*eg* patients' data, date of visit)
- reliability of results (*eg* collection of samples, microscopy reading, results)
- organization (*eg* stock management)
- maintenance (*eg* equipment, facility)
- surveillance (*eg* statistics, data analysis)

The above are evaluated using standard protocols, with the aims to ensure high quality and continuity of work. (Slama *et al,* 1993; Petithory and Foujade, 1994). Despite a general policy recommending the establishment of quality control programs in France, guided by the "Guide des Bonnes Executions des Analyses", many laboratories have suffered from lack of motivation for the set-up of such controls. For the success of such activities not only financial, but also team factors, such as adequate communication between team members and managers, have to be taken into consideration (Smith, 1979). In the future, well adapted and sustainable internal quality control program will hopefully become a reality (Handorf, 1994; Raymond *et al,* 1994; Howanitz and Howanitz, 1983).

Based on the results and observations made during this study, a number of recommendations can be made for the future : performance of specific training for national supervisors may help to optimize organization and functioning of the national teams. If national supervisors were brought to participate in the performance of the quality control examinations, supervisory visits of the international evaluator could be spaced even further apart and take place ad hoc rather than fixed intervals. We suggest that further research about the impact of performance of technic on results is needed.

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