Short Communication

External Quality Assessment: Microscopy Diagnosis Of *Plasmodium Falciparum* For A Better Management Of Malaria In The Regional Health Center In Côte D'ivoire

ABSTRACT

Context: In Côte d'Ivoire, malaria is transmitted throughout the year with an increased rate during the rainy season. This pathology is endemic on the whole territory with seasonal variations. The major vector is *Anopheles gambiae*. The external microbiology quality assessment programs organized by both Institut Pasteur of Côte d'Ivoire (IPCI) and PEPFAR, malaria microscopy was randomly carried out in 1/3 of the country regional health center laboratories. Laboratory technicians play a key role in malaria control programs because care services such as the disease monitoring depend on their diagnosis and technical skills.
Aim: The aim of this evaluation was to control the quality of the microscopic diagnosis and the performance of on-duty technicians for the management of feverish patients and efforts to strengthen laboratory services.
Méthodology: Six (6) RHC (Regional Health Center) laboratories were involved in the evaluation. Anonymity code was assigned to each of the participating laboratories.
There were many discrepancies in External Quality Assessment (EQA) results on the field not with standing the parasitemia, low or high.
Results: Only 30% of correct answers were recorded for *P. falciparum* identification. For P. ovale, we found a failure rate of 100% for laboratories.
Conclusion: Parasitemia was approximate and many confusions were observed regarding the different stages of parasites.

***Keywords*:** EQA, *Plasmodium*, parasitemia, microscopy, thick blood. *Plasmodium falciparum* Microscopy diagnosis, Côte d'Ivoire

1. INTRODUCTION

According to WHO last estimations published in December (2016), there were 212 million malaria cases and 429,000 deaths (2015). In Côte d'Ivoire the vulnerable population was estimated at 8 million (2015). 4 million cases of malaria were confirmed with 14 thousand 14,000 deaths recorded. The number of cases of malaria confirmed through microscopy was estimated to 500 thousand. All cases of diagnosed malaria were caused by *Plasmodium falciparum,* the most dangerous and dreadful plasmodial species and a good reduction in mortality depends inevitably on good management of malaria cases (Abrogoua *et al.,* 2006)
For an efficient treatment, WHO recommends that malaria is confirmed in all suspected cases by a diagnosis based on parasite research (by microscopy or rapid diagnostic test) prior to treatment. According to the National Malaria Control Program (NMCP) report published (2004) Not listed in reference, malaria represents 80% of medical consultations and hospitalisations in Côte d'Ivoire and accounts for 33% of mortality causes. In Côte d'Ivoire, malaria is transmitted throughout the year with an increased rate during the rainy season. This pathology is having a field in the form of stable malaria, endemic on the whole territory with seasonal variations. The major vector is *Anopheles gambiae* (Konan *et al*., 2008). Treatment only based on symptoms should not be considered if the parasitological diagnosis is not possible. Early diagnosis and treatment of malaria reduce disease intensity and prevent death. They also help reducing malaria transmission.
In a series of external microbiology quality assessment programs organized by both Institut Pasteur of Côte d'ivoire (IPCI) and PEPFAR, malaria microscopy was randomly carried out in 1/3 of the country regional health center laboratories. Laboratory technicians play a key role in malaria control programs because care services such as the disease monitoring depend on their diagnosis and technical skills.
The objective of this evaluation was to control the quality of the microscopic diagnosis and the performance of on-duty technicians for the management of feverish patients and efforts aiming at strengthening laboratory services. Several short courses of malaria through microscopy courses will significantly increase the knowledge and the level of microscopy skills of the trainees and will bridge up the significant difference in baseline microscopy skills of the different categories of trainees those

who participated in the courses.
For this purpose, six 6 laboratories of the Regional Health Centers (RHC) of the health districts of Côte d'Ivoire participated in the study of EQA in connection with the WHO guideline external quality assessment (WHO, 2014).

2. METHODOLOGY

Six (6) RHC (Regional Health Center) laboratories were involved in the evaluation. Anonymity code was assigned to each of the participating laboratories. It was about a Giemsa staining thick blood smears and thin blood film. The panel was made of twenty-four (24) thin blood film slides with the same staining. Thin blood films were made and stained 21 days before the expected date and results were validated by the National reference center for malaria chemo-resistance hosted by IPCI through the unit of malariology. Slide transportation was done by road and samples were given to the head of the medical analyzes laboratory.
A questionnaire was submitted to participants including slide code, clinical information about the patient, the result of thick blood smear with parasite density, the result of thin blood film with identification of species and sexual and non-sexual forms and results interpretation interpretation of results
Only the mean of parasitemia estimated by participants was calculated and reported. The results were saved as an Excel file. They were classified as: correct (parasitic density inferior or equal to 10%), minor errors (non-significant difference or parasitic density inferior or equal to 20%) or major errors (incorrect diagnosis on species or sexual or non-sexual forms or Incorrect interpretation or parasitic density exceeding 20%).

3. RESULTS AND DISCUSSION

Many discrepancies were noticed in the parasitic microscopy results for both the density and determination of species. Some results were over estimated (table 1) and others were underestimated by participants (Table 1). Only 30% of correct answers were recorded for ***P. falciparum*** ***unbold*** identification. For *P. ovale*, we found a failure rate of 100% for laboratories (Table 2). Parasitemia was approximate and many confusions were much confusion was observed regarding the different stages of parasites (table 1). However, there is a laboratory (lab. 007) which from the point of view of parasitological diagnosis and diagnosis of species has nothing found. Some technicians could use today the so-called "plus system" which is an old, simple, but much less precise method for establishing parasitic density in thick blood smears. Because of its unreliability, it was replaced by the method of determination of parasitic density by calculation, a simple mathematical formula, which multiplies the number of parasites by 8000 (standard number of leukocytes/µl) dividing by the number of leukocytes (200 or 500). The result is the number of parasites/µl of blood. Studies showed that many technicians forgot the details of the plus system and were mistaken about the code (the number of signs +) and numeration (the number of parasites per field or for 100 fields), which leads to unreliable information about the parasitic density. The number of red blood cells infected with ***P. falciparum*** ***unbold*** parasites is essential and the percentage of parasitemia should always be reported as this has effects on the prognosis and the mode of treatment used. This qualitative assessment may be considered insufficient in malaria-endemic areas (Benasseni *et al.,* 1987; Baudon *et al.,* 1988). But some biologist technicians who do not want to get rid of this method would have difficulty adapting to the counting methods by in force.
Overestimation of parasitemia observed in some participants could be due to counting errors. A red blood cell infected with multiple parasites counts as a parasitic red blood cell. Another reason could include gametocytes when calculating parasitemia. When calculating the *Plasmodium falciparum* parasitemia, only the trophozoite stages are were counted. Gametocytes and other species of malaria parasites are excluded from the result, but it was important to know them and to differentiate all forms of the parasite biomass (Table I and II). Participants who underestimated parasitemia might not have counted a sufficient number of fields (Table I). It is recommended to count 40 fields of a thin blood smear (Table II), and Thick films should be examined by two observers, each viewing 200 high power fields or counted 200 leukocytes. Especially when parasitaemia is low due to possible unequal distribution of parasites or to count in the case of a thick blood smear, it is recommended to Count

until 500 leukocytes (WHO, 2009). Considering the diagnostic results per laboratory, no laboratory scored less than 80%, level of technical skill and accuracy expected for the examination of a series of slides for accreditation is inevitable, whereas the scores of all assessed laboratories range from 0% to 50% (fig1). Technicians, once the certificate is obtained once certified, it is important to ensure that the level reached during the training is maintained. To achieve this, it is agreed that their work is regularly monitored by a supervisor at all times to help them improve their techniques and skills. This is called quality control which is part of the general activities of quality assurance applied in all microscopic malaria diagnostic services (WHO, 2010).

Table 1: Analysis of laboratory performance according to parasitemia results

Order number of assessed Lab	***Plasmodium strain* Slide 1**		***Plasmodium strain* Slide 2**		**Assessed Lab results categorization**	
	NRC Lab	**Assessed Lab**	**NRC Lab**	**Assessed Lab**	**Slide 1**	**Slide 2**
001	440 gam/µl	2400 tr/µl	4440 sch/µl	4800 tr/µl	Major error	Major error
002	7080 tr/µl	3600 tr/µl	1600 sch/µl	Nc	Major error	Major error
003	840 gam/µl	Nc	20120 tr/µl	8500 sch/µl	Major error	Major error
004	1638 tr/µl	1700 tr/µl	4120 sch/µl	Nc	Correct	Major error
006	360 gam/µl	600 tr/µl	2400 sch/µl	Nc	Major error	Major error
007	850 gam/µl	Nc	3890 sch/µl	Nc	Major error	Major error

gam: gametocyte; tr: trophozoïte; sch: schizonte
Nc: Not counted
NRC: National Reference Center
Lab: laboratory

Table 2 Laboratory performance analysis according to the results of the identification of plasmodial strain

Order number of assessed Lab	***Plasmodium strain* Slide 3**		***Plasmodium strain* Slide 4**		**Assessed Lab results categorization**	
	NRC Lab	**Assessed Lab**	**NRC Lab**	**Assessed Lab**	**Lame 3**	**Lame 4**
001	*Pf*	*Pf*	*Po*	*Pm*	Correct	Major error
002	*Pf*	*Po*	*Po*	Ni	Major error	Major error
003	*Pf*	Ni	*Po*	*Pf*	Major error	Major error
004	*Pf*	*Pf*	*Po*	Ni	Correct	Major error
006	*Pf*	*Po*	*Po*	Ni	Major error	Major error
007	*Pf*	Ni	*Po*	Ni	Major error	Major error

Pf : *Plasmodium falciparum* ; *Po* : *Plasmodium ovale* ; *Pm* : *Plasmodium malariae*
Ni : Nothing identified

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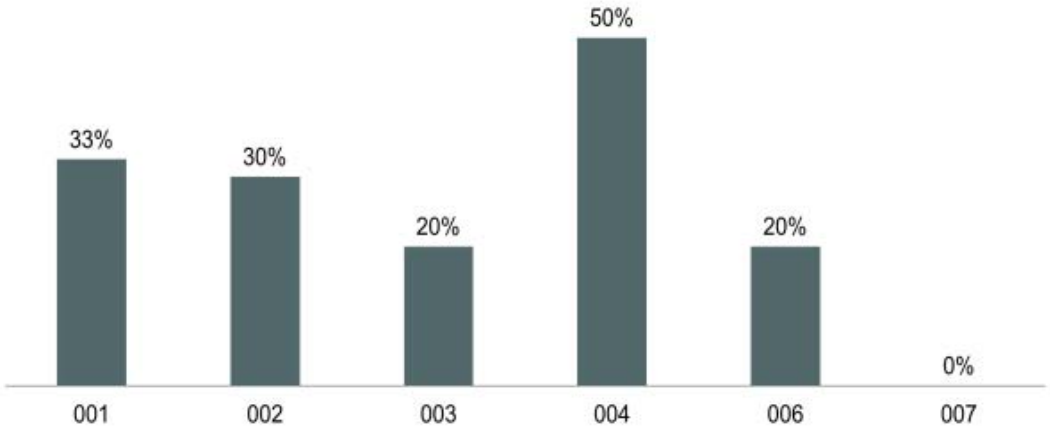

Figure 1 Mean obtained by participating laboratory

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4. CONCLUSION

Microscopy identification of parasite though being WHO standard method opens up a current reflection for a better management of malaria. Rapid diagnostic tests (RDTs) for a malarial antigen cannot replace microscopy but are indicated as a supplementary test when malaria diagnosis is performed by relatively inexperienced staff. Microscopy requires a high qualified staff which is not always available in areas where malaria is endemic. It is therefore important to maintain the level reached by technicians during their training. This requires supervision and a regular supervision of their work. This will help to continuously improve their skills. Quality control should be part of the general activities applied in all microscopic malaria diagnostic services.

COMPLIANCE WITH ETHICAL

This study was carried out according to the guidelines of the Ivorian National reference center for malaria chemo-resistance created by the interministerial decree number 393/08/ 2006, and conduct research according to the Ivorian National Ethical Committee and Research with due approval. Therefore, this study was performed after receiving approval from the Ivorian National Ethical Committee and Research.

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