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Factors associated with non-compliance to the pre-analytical phase of the malaria thick blood smear in the Lemba Health Zone, Democratic Republic of Congo

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Abstract

Introduction: The medical laboratory is an important link in the process of patient care; it guides the practitioner to make a diagnosis or prognosis of diseases, especially to ensure therapeutic follow-up. Laboratory results contribute from 31% to 85% in the establishment of the diagnosis. The study determined the factors associated with non-compliance in the pre-analytical phase of the thick blood smear for malaria in the health facilities of the Lemba health zone.

Methods: A cross-sectional study was conducted in 8 laboratories of the Lemba health zone. The statistical units were the health facilities, the analysis requesters, the request forms and the laboratory technicians. The chi-square test allowed us to determine the factors associated with the non-compliance of the pre-analytical phase of the thick drop.

Results: The frequency of thick drop requests was 65% of the total number of analysis request forms issued, nearly six out of ten test vouchers were non-compliant, and more than six out of ten pre-treated samples were non-compliant. Three factors were associated with non-compliance in the pre-analytical phase of the thick drop: the nursing category (cOR=12.5; 95% CI: 5.61-28.00), lack of training of laboratory technicians on how to perform the thick drops (cOR=16.0; 95% CI: 1.60-159.31), and lack of standard operating procedure (cOR=9.6; 95% CI: 4.72-19.59)

Conclusions: As the thick drop is a reliable technique for the biological diagnosis of malaria, it is very important that its pre-analytical phase conforms to ISO 15189 standards, in order to guarantee the reliability of laboratory results and enable clinicians to guide their approach to the medical management of malaria patients.

Keywords: Factors associated, Thick blood smear for malaria, Health zones

Citation

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Introduction

The pre-analytical phase, which some consider to be the gateway to the quality assurance system in the biological laboratory, can be defined as a series of steps that include the ordering of tests by the clinician, preparation of the patient by the intake department, sample collection, delivery to the laboratory, and pre-treatment of the sample [1-3]. The medical biology laboratory is of paramount importance as an important link in the patient management process, in that it guides the practitioner in making the correct diagnosis or prognosis of diseases and, above all, in ensuring therapeutic follow-up. For example, laboratory test results contribute 31% to 85% in the establishment of a diagnosis [4-12].

The application of the ISO 15189 standard imposes the duty on each laboratory to define its pre-analytical compliance, in particular the instructions relating to the methods for collecting, transporting, storing and pre-treating samples before analysis. As the thick drop is a reliable technique for the biological diagnosis of malaria, it is very important that its pre-analytical phase conforms to ISO 15189 standards [6]. The conformity of laboratory analyses implies the control of the pre-analytical phase, as it is an important condition for the quality of the result and thus the accuracy of the diagnosis and/or the therapeutic evaluation. The sources of non-compliance during this phase can be multiple since it involves different locations and several stakeholders [3].

In most parts of Africa, it is common practice to diagnose malaria based on symptomatology alone. However, the clinical diagnosis of malaria is highly imprecise, as symptoms are non-specific and may be the manifestation of other febrile infectious diseases. For effective management of malaria, a correct diagnosis must be made without delay. Clinical suspicion of malaria and the detection of haematozoa in the blood allow a parasitological or confirmatory diagnosis to be made[4].

Correct management of malaria cases requires early diagnosis and prompt treatment with effective antimalarial drugs. This is one of the basic strategies in the fight against malaria in the Democratic Republic of Congo (DRC). It remains an essential component of malaria control strategies. It involves rapid diagnosis and early, correct and effective treatment of the disease with effective antimalarial drugs [5]. Biological confirmation by RDT or microscopy (thick drop, thin smear) is a mandatory recommendation before starting treatment in all patients for whom there is clinical suspicion of malaria, at all levels of the health system, including the community level [5].

ISO 15189 requires periodic verification of the malaria thick smear/blood smear accuracy through participation in national or international external quality assessment (EQA) programs of all three phases (pre-analytical, analytical and post-analytical). Each phase requires special attention, as it has a major influence on the conformity or quality of the result, and hence on the diagnostic and therapeutic management of the patient [6].

The thick drop test of malaria is more sensitive than other methods, such as the thin blood smear, meaning that it can detect parasites even at low concentrations. Results can be obtained rapidly, often within an hour of blood sampling, which is crucial for rapid and effective treatment. Secondly, it is often used in emergencies to rapidly confirm a diagnosis of malaria, especially in endemic areas [7].

In the Democratic Republic of the Congo, although there is a national laboratory network and many schools that train laboratory technicians, to our knowledge, we did not find any studies conducted on factors associated with non-compliance in the pre-analytical phase of the malaria thick blood smear. The Lemba health zone performed 19011 thick drops of malaria during the year 2018 with a positivity rate of 80.6% [13]. Compare this 80.6% malaria positivity rate with the 18% malaria positivity rate found in the demographic and health survey in Kinshasa [14]. We believe that the thick drop test results returned by the laboratories in the Lemba health zone did not reflect reality. The overall objective of this study was to determine the factors associated with the non-compliance of the pre-analytical phase of the malaria thick drop test in the laboratories of the Lemba Health Zone.

Methods

Study setting, design and sample size

The Lemba health zone was the only health zone considered due to its high prevalence of malaria compared with the rest of the other health zones in the city of Kinshasa. A cross-sectional analytical study was conducted in eight health facilities in the Lemba Health Zone. The sample size was calculated as follows [10]:

$$n \geq \frac{Z_\alpha^2\, p(1-p)}{d^2} = \frac{(1.96)^2 \times (0.87 \times 0.13)}{(0.05)^2} = 174$$

n: the sample size

Zα: The Z value corresponding to the desired confidence level

p: Proportion of pre-treated samples in compliance, we considered a prevalence of 87%/ [15].

q: Proportion of non-compliant pre-processed samples

d: the desired margin of error or precision expressed as a proportion

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Sampling technique

A total of 23 health facilities were eligible, including two hospitals. These two hospitals were purposively included in the study, as well as six other health facilities selected by sampling proportional to the sector of belonging (3 public, 3 private and 2 denominational establishments) and the type of health facility (one hospital, two clinics, one reference health center, three health centers and the University Clinics of Kinshasa). Thus, the survey was conducted in a total of eight health facilities, which represented 35 percent of all health facilities in the Lemba health zone. In each health facility, all vouchers for the thick test that arrived at the laboratory within two days of the collection agents' visit, as well as the applicants for these vouchers, were included in the study. In addition, all laboratory technicians responsible for pre-treating samples for these vouchers were also included in the study.

Operational definitions

Application form compliance: The thick test requisition form was considered compliant when it contained the following information: patient entry number, patient's first and last name, applicant's signature, nature of test requested, gender, patient's age, and date when the test was ordered[11, 16]. If any of these elements were missing, the examination request form was considered non-compliant. The frequency of malaria thick smear requests is the proportion of malaria thick smear requests among all requests sent to the laboratory.

Pre-treatment sample compliance: The sample compliance index was determined by calculating the percentage score obtained for all 18 components (1: component is achieved, 0: component is not achieved). It was obtained by adding the score for each component, dividing by 18 and multiplying by 100. This index made it possible to make an overall assessment of sample compliance for each observation. This overall assessment of the level of compliance had three categories: good, medium and poor. A sample was considered to have a good compliance level if it attained a maximum score of 80% or higher. Otherwise, it was considered medium compliance for a maximum score of 60% to 79.9% and poor compliance for a maximum score of below 60%. A pre-treatment sample was considered non-compliant at both medium and poor compliance levels.

Statistical analysis

The data was coded in Excel 2013 and then exported to SPSS version 23 for analysis. Frequency measures were calculated using proportions for the categorical variables. Bi-variate analysis helped us to identify factors associated with non-compliance from the pre-analytical phase at a significance level of $\alpha = 0.05$. Our study is also limited by the fact that, during the bivariate analysis, we did not include independent variables such as hospital type and affiliation in order to investigate associations between these variables and the level of compliance of the sample and the application form. Thus, we did not perform logistic regression in this study, which is a significant limitation, as it prevents us from quantifying the independent association between the explanatory variables and the outcome studied. It is also difficult to control for potential confounding factors, which may bias the interpretation of the results.

Ethical consideration

We obtained the authorisation of the ethical committee of the health zone of Lemba (017/19/ZSLEM) prior to conducting the study. We obtained the consent of study participants, and participants were free to choose to take part or not without any repercussions for opting out of the study. We respected anonymity and confidentiality through the coding of identifying data, the completion of data entry by the research team and the restriction of access to data by the research team. Participants did not run any major risk and had no direct benefits to expect.

Results

A total of eight health facilities, 178 thick test requisition vouchers, 178 samples related to these vouchers, 27 laboratory technicians and 37 thick test applicants were included in this study. Of these eight health facilities, there were three health centres, one referral health centre, and two clinics, of which three out of eight were public health facilities and two out of eight were church health facilities.

Table 1. Overall compliance of different phases of pre-analytic thick blood smear for malaria (N = 178)

Variables	n	%	95% CI
Level of compliance with pre-analytical thick smear sample processing			
Good	63	35.4	(28.3–42.8)
Medium	52	29.4	(22.6–36.4)
Poor	63	35.4	(28.3–42.8)
Overall sample compliance			
Non-compliant	115	64.6	(57.1–71.6)
Compliant	63	35.4	(28.3–42.8)
Level of compliance in completing the requisition form			
Non-compliant	104	58.4	(50.8–65.7)
Compliant	74	41.6	(34.2–49.1)

In total, there were 274 requests for examination, of which 178 were for thick malaria drops, representing a proportion of 65% (178/274) of the request slips issued in malaria laboratories. Nearly six out of ten laboratory technicians (16/27) were women, and were aged between 19 and 34. In total, we had 27 people pre-processing the samples, of whom 74% (20/27) were laboratory technicians, 22.2% (6/27) were academic trainees, and 3.7% (1/27) was a nurse, 63% (17/27) were graduates, and 55.5% (15/27) had received no on-the-job training in thickness testing techniques.

With regard to the gender of applicants for thick malaria smears, a slight majority are male (20/37), representing

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Table 2. Relationship between applicant characteristics and non-compliance with completing the thick blood smear requisition form (N = 178)

Variables	Non-compliance with completing requisition form		cOR	95% CI	p-value
	Yes (n=104) n (%)	No (n=74) n (%)			
Sex					
Male	76 (73.1)	55 (74.3)	0.93	(0.47–1.84)	0.852
Female	28 (26.9)	19 (25.7)	1	–	–
Age (years)					
19–34	57 (51.9)	32 (43.2)	1.59	(0.87–2.90)	0.128
35 and over	47 (45.2)	42 (56.8)	1	–	–
Professional cadre					
Nurse	66 (63.5)	9 (12.2)	12.54	(5.61–28.00)	<0.001
Doctor	38 (36.5)	65 (87.8)	1	–	–
Number of years in service					
Below 5	54 (51.9)	35 (47.3)	1.20	(0.66–2.18)	0.542
5 years and older	50 (48.1)	39 (52.7)	1	–	–

54.1% of the total, while 45.9% (17/37) are female. The most represented age group is those 43 years of age and above (21/37), representing 56.8%, while those under 43 years of age represent 43.2% (16/37). In terms of professional category, the overwhelming majority of applicants are doctors, with 29/37 individuals representing 78.4% of the group. In contrast, only 8/37 are nurses, representing 21.6%.

Analysis of length of service shows that 19/37, or 51.4%, have less than 8 years' experience in the service, while 18/37, or 48.6%, have 9 years or more. All health facilities had sample traceability records, and nearly four out of ten health facilities had Standard Operating Procedures (3/8). None of the facilities had a quality manager or supervision.

Nearly six out of ten test orders (58.4%, 104/178) were non-compliant in completing the requistion form (Table 1). Non-compliant test orders were statistically significantly associated with being written by a nurse compared to compliant orders (64% vs 12%; cOR=12.5; 95% CI: 95%: 5.61-28.00). This difference was also practically significant (Table 2).

Sixty-five per cent of the pre-processed samples (115/178) were non-compliant. Three factors were associated with non-compliance of the malaria thick drop pretreatment samples. The fact that a request form is issued by a nurse instead of a doctor is associated with non-compliance with request forms for thick smear malaria. (63.5% versus 12.2%; cOR = 12.54; 95% CI: 5.61-28.00), The fact that a laboratory technician who has not been trained on the job in good practices for sample pre-treatment for malaria thick smears is associated with non-compliance in the pre-analytical phase of malaria thick smears. (66.6% vs 11.1%;cOR=16.0; 95% CI: 1.60-159.31) The fact that a healthcare facility does not have standard operating procedures for performing thick smear microscopy for malaria is associated with non-compliance in the pre-analytical phase of thick smear microscopy for malaria (81.7% vs 31.7%; cOR=9.6; 95%CI: 4.72-19.59). Notably, the associations of lack of on-job training and lack of SOPs with non-compliant samples also had practical significance (Tables 2 and 3).

Discussion

Thick smear testing is an essential diagnostic test for malaria, as it detects the presence of parasites in the blood. Its practical importance lies in its ability to provide a rapid and accurate diagnosis, which is crucial for initiating appropriate treatment and reducing the morbidity and mortality associated with this disease.

Adherence to standard operating procedures (SOPs) when performing thick smears is fundamental to ensuring reliable results. By following these procedures, laboratories can ensure accurate diagnoses, which is essential for epidemiological surveillance and malaria case management. The frequency of demand for the thick test is higher compared to other demands, with 65% of the total demand, which could be due to the endemicity of malaria in the area.

Our study shows that a considerable number of vouchers examined (about six out of ten) were non-compliant. This may be explained by the fact that most of the vouchers are issued by nurses rather than doctors, which is contrary to the recommendation of ISO 15189 for the medical prescription sheet [17]. The result of the present study is not in agreement with another study conducted in Rabat in 2012, which found 32% non-compliance related to the request forms [2].

Overall, 45.5% of laboratory technicians did not receive on-the-job training on the pre-analytical phase of the thick test, which is contrary with the result found by S. Beldjilali and Al in a study conducted in the People's Democratic Republic of Algeria in 2018. Nearly 58% of the respondents declare that they have received training on the

Table 3. Factors associated with non-compliance with the pre-analytic thick blood smear sample processing (N = 178)

Variables	Non-compliance with pre-analytic sample processing		cOR	95% CI	p-value
	Yes (n=115) n (%)	No (n=63) n (%)			
Sex					
Male	70 (60,9)	32 (50,8)	1.5	(0.81–2.80)	0,193
Female	45 (39,1)	31 (49,2)	1	–	–
Availability of SOP*					
No	94 (81,7)	20 (31,7)	9.6	(4.72–19.59)	<0,001
Yes	21 (18,3)	43 (68,3)	1	–	–
Level of education of laboratory technicians					
Graduate	13 (72,2)	6 (66,7)	1.3	(0.23–7.31)	0,544
Intern	5 (27,8)	3 (33,3)	1	–	–
On-the-job training					
No	12 (66,6)	1 (11,1)	16.0	(1.60–159.31)	<0,001
Yes	6 (33,3)	8 (88,8)	1	–	–
Age (years)					
19–34	11 (61,1)	3 (33,3)	3,14	(0.58–16.84)	0.05
35 and more	7 (38,9)	6 (66,7)	1	–	–

*SOP: Standard Operating Procedures

pre-analytical phase [10].

In the present study, the results show that 100% of the health facilities surveyed had a traceability register, which agrees with the results found in another study conducted in 2015 in France, which found that 95% of laboratories had a traceability register [13].

The result found in this study shows that no health care facility had a quality manager or supervision, which converges with the result of the survey conducted by the National Strategic Plan for the Development of the Laboratory System 2011-2015. The survey revealed that the quality management system for laboratory services is not implemented in all laboratories in the Democratic Republic of Congo. While the quality system requires the laboratory to have a manager to oversee the management of the quality system within the laboratory.

The present study showed that only 36% of the health facilities had standard operating procedures, whereas the microscopic diagnosis of malaria is a technical examination, requiring great care at each step of the standard operating procedures, as well as visual and analytical skills[18]. The absence of standardised laboratory procedures can lead to variations in the quality of diagnoses, which is particularly critical in the case of malaria, where early and accurate diagnosis is essential for effective treatment. Thus, this can have direct consequences on the management of malaria in communities, increasing the risk of complications and death[19].

Our study also showed that 6% of the pre-treated samples were non-compliant, while another study in Rabat found that 3.9% of the pre-treated sample was non-compliant [13]. The high prevalence of non-complaint samples in our study could be explained by the lack of supervision or on-job training of laboratory staff. Mabey, D et al point out in their article that in many health facilities in Africa, a significant percentage of samples for malaria diagnosis fail to meet the required standards, which can lead to misdiagnosis and inappropriate treatment, highlighting a systemic problem that requires urgent attention [20].

The issuance of a request form by a nurse is significantly associated with non-compliance with forms for thick malaria smears, with an odds ratio of 12.54, indicating that this process could introduce errors into the diagnostic chain [21]. Studies show that involving doctors in requesting tests improves the accuracy and compliance of forms [22, 23].

The lack of field training for laboratory technicians in the pre-processing of thick malaria smear samples is strongly associated with non-compliance in the pre-analytical phase, as indicated by the results with an odds ratio of 16.0 (95% CI: 1.60-159.31). These results highlight the importance of adequate training to ensure the quality of parasitological diagnoses [22, 23]. The absence of standard operating procedures (SOPs) for performing thick malaria smears is strongly correlated with non-compliance in the pre-analytical phase, with an odds ratio of 9.6, highlighting the importance of SOPs in ensuring the quality of diagnoses [24]. One study has shown that the implementation of SOPs reduces diagnostic errors, thereby improving the reliability of results [16].

Limitations

A limitation of our study is that samples taken outside the collection service were not taken into account, yet there may have been some non-compliers who may be the cause

of a misdiagnosis. Nevertheless, this study is the very first in the Democratic Republic of Congo and particularly in Kinshasa, taking into account the available information. The results of this study may inspire health and political authorities at all levels to fight against non-compliance related to the pre-analytical phase of thick test in the city province of Kinshasa. Our study is further limited by the fact that during bivariate analysis, we did not include independent variables of hospital type and affiliation, in order to search for associations between the latter and the level of compliance of the sample and the request form. Thus, we did not perform logistic regression in this study, which is a significant limitation, as it prevents us from quantifying the independent association between the explanatory variables and the outcome studied. It is also difficult to control for potential confounding factors, which may bias the interpretation of the results.

What is already known about this topic

- The distribution of the non-conformities of the laboratory examinations showed that 85% of the identified non-conformities emanate from the pre-analytical phase, while the non-conformities revealed during the analytical and post-analytical phases are respectively 4% and 11% of the total non-conformities studied.
- The distribution of non-conformities related to the different stages of the pre-analytical phase, shows a dominance of non-conformities related to transport which constitutes about 56%, followed by non-conformities related to the prescription with 27%.
- The non-conformities of the pre-analytical phase result first of all from the major lack of coordination between the laboratory and the services, then from the low competence of the personnel involved in this process and, finally, from the absence of manuals for sampling, packaging, transport, reception and sorting.

What This Study Adds

- The frequency of requesting thick malaria test is higher compared to other requests accounting for 65% of total requests out of which 64.4% of the pre-processed sample were non-compliant.
- Factors associated with non-compliance in the pre-testing phase of malaria thick smear were identified as follows: nursing category, lack of standard operating procedures, lack of in-service training.
- None of the health care facilities had a quality manager and no supervision, so quality management of laboratory services was not implemented at all in all the laboratories surveyed.

Conclusions

As the thick drop is a reliable technique for the biological diagnosis of malaria, it is very important that its pre-analytical phase conforms to ISO 15189 standards, in order to guarantee the reliability of laboratory results and enable clinicians to guide their approach to the medical management of malaria patients. In light of the results obtained from this study, we recommend the following:

- Strengthen the capacity of laboratory technicians to perform thick drop tests for malaria,
- Assign a quality manager to each laboratory, to improve laboratory management of non-conformities,
- Make standard operating procedures available at laboratory level,
- Integrate laboratory supervision as a routine activity.
- Post standard operating procedures and follow all steps when pre-processing samples.
- Don't leave the burden of sample pre-treatment to trainees.

Conflict of Interest

The authors of this work declare no competing interests.

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Authors' contributions

Fidèle Djamba Okitokonda: design, literature compilation, interpretation and writing; Ernest Ombha Loshima: data collection and data entry; Guy Bilulu Suama: data collection and data entry; Freddy Kambale Kavoga: database design; Éric Panzi Kalunda: Correction and interpretation; Pélagie Babakazo: orientation and supervision.

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