Quality assessment of malaria microscopic diagnosis at the LPM-HALD of Dakar, Senegal, in 2020

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Abstract

Background: Following WHO guidelines, microscopy is the reference for malaria diagnosis in endemic countries. The Parasitology-Mycology laboratory (LPM) is the National Reference Laboratory and is currently undergoing ISO 15189 accreditation. In this perspective, we proceeded to laboratory verification through assessing the performance of the laboratory by confirming the reliability and the accuracy of the results obtained in accordance with the requirements of the ISO 15189 standards. This study aimed to verify the method of microscopic diagnosis of malaria at the LPM, in the Aristide Le Dantec hospital (HALD) in Dakar, Senegal.

Methods: This is a validation/verification study conducted from June to August 2020. Twenty (20) microscopic slides of thick/thin smear with known parasite densities (PD) selected from the Cheick Anta Diop University malaria slide bank in Dakar were used for this assessment. Six (6) were used to assess readers' ability to determine PD and fourteen (14) were used for detection and identification of parasites. Four (4) LPM-HALD microscopists read and recorded their results on prepared sheets. Data analysis was done with Microsoft Excel 2010 software.

Results: Of these twenty (20) slides read, a 100% agreement was obtained on eight (8) slides. Four (4) out of the six (6) parasite density evaluation slides obtained a concordance of less than 50%. Thirteen (13) out of the fourteen (14) identification slides obtained a concordance greater than 50%. Only one (1) identification slide obtained zero agreement from the readers. On the other hand, for species identification it was noted a score greater than 80% and the PD obtained a score between 0.20 and 0.4. Readers obtained 100% precision, sensitivity, specificity and both negative and positive predictive values.

Conclusion: This work demonstrated that the microscopic method of malaria diagnosis used in the LPM/HALD are in accoradance with all the reliability required by ISO 15189.

Keywords: Malaria, *Plasmodium*, Microscopy, Quality, Reliability, Senegal.

Background

In Senegal, the National Malaria Control Program (NMCP) and its partners have adopted control strategies for the elimination of malaria. They contributed to registering a significant reduction of more than 50% of the disease burden between 2009 and 2015 with a parasite prevalence which decreased from 3% to 1.2% and mortality from 72‰ to 33‰ among children less than 5 years [1–3].

These strategies include laboratory diagnosis, which emanates from the WHO recommendation that only confirmed cases should be treated with ACTs. In endemic areas, RDTs and light microscopy are the most widely used and the latter constitutes the reference technique according to WHO recommendations [4–8].

Microscopy is available in intermediate, central and peripheral health facilities or rural health centers. However, the quality of this microscopic diagnosis is essential to guarantee an adequate treatment in order to maintain this trend of reduction in morbidity and mortality linked to malaria. Therefore, the effectiveness of malaria microscopic diagnosis remains dependent respectively on maintaining a high level of staff skills and performance, the availability of good quality reagents and equipment at all levels, and regular internal and external evaluations [5, 7, 9–11].

The Parasitology and Mycology Laboratory of the Aristide Le Dantec Hospital (LPM/HALD) is enrolled into an accreditation process through the West African Society of Accreditation and Certification according to ISO 15189 version 2012 [13]. Thus, using microscopic method of malaria diagnosis, it must demonstrate the performance of the laboratory system, confirm the reliability and accuracy of the results obtained and ensure continuous improvement of the Quality Management System.

Indeed, the ISO 15189 Standard is an accreditation standard which presents general requirements concerning the quality and competence of Biomedical Laboratories. Among the requirements of this standard for accreditation is the verification and the validation of the methods used for diagnosis. Here, we will proceed to the verification by demonstrating the performance of the laboratory system, by assessing the reliability and the accuracy of the results obtained in accordance with the requirements from the ISO 15189 standard [13, 19].

The objectives were to determine the concordance of the results obtained between readers, to measure the indicators of qualitative evaluation of the microscopic diagnosis of malaria according to the verification parameters of a qualitative method, and to estimate the sensitivity and specificity as well as the indices of predictive values of the different readers.

Methods

The Laboratory of Parasitology and Mycology at Aristide Le Dantec Hospital in Dakar, Senegal [**Figure 1**], is the National Reference Laboratory for malaria in Senegal and as such supports the NMCP in the microscopic diagnosis of malaria. This laboratory is also involved, under the agreement of WHO and the NMCP, in the training and accreditation of African experts in microscopy.

Type and period of study

This is a verification assessement study conducted from June to August 2020.

Study sample

Slides of thick smears and thin smears made and stained [**Figure 2**] by LPM-HALD with known parasite densities were chosen from the slide bank of Cheick Anta Diop University (UCAD). One (1) of these slides was repeated three times. The slides were validated by the WHO Level 1 expert microscopists in Senegal and by real-time PCR at UCAD.

Sample size

Twenty (20) slides were examined in accordance with WHO recommendations as part of microscopist certifications using the WHO competence levels and criteria [**Table I**] [2, 3]. For the general characteristics of the evaluation slides [**Table II**], a total of twenty (20) slides were read by four (4) different readers. Of these twenty (20) slides, six (6/20) positive thick smears were used to assess the ability of the readers to determine the parasite density (PD) and the other fourteen (14/20) were used for parasites identification. Of these fourteen (14), eight (8) were positive with at least one species of *Plasmodium* and six (6) were negative with no parasite.

Description of variables and data collection

According to the on-site verification of the performance of a method in the technical guide for the accreditation of methods in medical biology of the standard, four (4) microscopists ensuring the schedule of on-call services at the LPM/HALD each read and recorded the results of the reading of the twenty (20) mixed thick smear slides using the internal competency assessment form for malaria microscopists in Senegal [Supplementary] also called “collection sheet” prepared by the slide bankat the LPM-HALD. The aim was to identify *P. falciparum* and other species responsible for malaria after reading the slides using the 100X oil Immersion objective lens on a microscope [Figure 3]. The determination of the parasite density on tin smear in case of positive slide was made according to the following formula [6, 13, 19]:

$$\mathbf{PD} = \frac{\mathbf{number\ of\ parasites\ counts}}{\mathbf{number\ of\ WBC\ counts}} \mathbf{x\ 8000}$$

* PD: Parasite Density: nomber of parasites/µl ou mm^3 of blood (corresponding to 8000 WBC)
* WBC: White Blood Cells (200 WBC for a thick smear with high PD and 500 WBC for a thick smear with low PD).

The determination of the **scores** obtained by the participants was carried out using the WHO method which corresponds to the number of correct results [identification and PD] on the total number of slides read [6, 11].

The determination of the **concordance** between the slide readers was carried out according to the WHO method corresponding to the common results obtained by the readers and which agree with the reference [7, 12].

Inter-operator variability/concordance of a qualitative method

The inter-operator variability constitutes an indicator of the control of the realization of the non-automated methods. The laboratory will be able to use the inter-operator variability and compare it to the intra-operator variability of a referent.

Another possibility to quantify the inter-operator variability will be the analysis of variance applied to the results obtained by the n operators. Likewise, agreement can be used to measure inter-operator variability [13, 15].

ISO 5725 uses two terms "accuracy" and "precision" to describe the accuracy of a measurement method. "Trueness" refers to the closeness of agreement between the arithmetic mean of a large number of test results and the true or accepted reference value. A qualitative method will be all the more accurate if the values obtained are close to 100 when they are expressed in% [13, 16, 17].

The precision

The precision is, according to ISO 3534-1, “the closeness of agreement between independent test results obtained under specified conditions.

The mathematical definition is:

$$Precision\ (\%) = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FP}} x\ 100$$

A qualitative method will be all the more precise as the values obtained will be close to 100 when they are expressed in percentage (%) [13, 16, 17].

The accuracy

The accuracy is, according to ISO 3534-1, the closeness of agreement between the mean value obtained from a large series of test results and an accepted reference value.

Microscopy performance measures the correctness of the results [accuracy of diagnosis and report] of the microscopist in everyday practice [12, 13, 18].

The mathematical definition is:

$$Accuracy\ (\%) = \frac{\mathrm{TP} + \mathrm{TN}}{\mathrm{TP} + \mathrm{TN} + \mathrm{FP} + \mathrm{FN}} x\ 100$$

Sensitivity (Se) and Specificity (Sp)

According to the technical guide for accreditation of verification (scope A)/validation (scope B) of methods in medical biology, the concepts of sensitivity and specificity are used for dichotomous tests (yes or no, positive or negative, etc.). The sensitivity and specificity of a test give an appreciation of its intrinsic validity [13, 19]:

The **Sensitivity** is also called Fraction of True Positives which is the proportion of positive individuals actually detected by the test. In other words, sensitivity is a measure of how well the test performs when used on positive individuals. The test is perfect for positive individuals when the sensitivity is 1, equivalent to a random draw when the sensitivity is 0.5. If it is less than 0.5, the test is counter-performing and it would be in your interest to reverse the rule so that it is greater than 0.5 (provided that this does not affect specificity). Thus, in the case of microscopy, it is the proportion of slide readers who detected positive slides knowing that the slides do indeed contain malaria parasites.

The mathematical definition is:

$$\text{Sensitivity}\ (\%) = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FN}} x\ 100$$

The **Specificity** is also called the True Negative Fraction which is the proportion of negative individuals effectively detected by the test. In other words, the specificity measures how well the test performs when used on negative individuals. The test is perfect for negative individuals when specificity is 1, equivalent to a random draw when specificity is 0.5. If it is less than 0.5, the test is counter-performing and it would be in your interest to reverse the rule so that it is greater than 0.5 (provided that that does not affect the sensitivity). Thus, for the case of microscopy, it is the proportion of slide readers who detected negative slides knowing that the slides do not contain a malaria parasite.

The mathematical definition is:

$$\text{Specificity}\ (\%) = \frac{\mathrm{TN}}{\mathrm{TN} + \mathrm{FP}} x\ 100$$

The positive predictive value (PPV) is the probability that the disease is present when the test is positive [13]. So, applied microscopy, this is the probability that the parasite is present when the slide is actually positive.

The mathematical definition is:

$$\text{PPV (\%)} = \frac{\text{TP}}{\text{TP} + \text{FP}} x\ 100$$

The negative predictive value (NPV) is the probability that the disease is not present when the test is negative [13]. For the case of microscopy, the NPV is the probability that the parasite is absent when the slide is actually negative.

The mathematical definition is:

$$\text{NPV (\%)} = \frac{\text{TN}}{\text{TN} + \text{FN}} x\ 100$$

Statistical analysis

Data were entered, coded, rechecked and analyzed using Microsoft Excel 2010 software. [16, 18]

Results

Concordance of results obtained by slide readers/microscopists

During this study, the value of the agreement varied between slides. Thus, on the twenty (20) slides read, a 100% agreement was obtained on eight (8/20) slides [**Figure 4**]. Four (4) of the six (6) parasite density slides it was noted a concordance of less than 50% [**Figure 5**]. Thirteen (13) of the fourteen (14) identification slides obtained a concordance greater than 50% [Figure 6]. Only one (1) identification slide met with no concordance from slide readers [Figure 7].

Reader's skill level: scores compared with references

A score greater than or equal to 80% on the identification of the species compared to the reference and a score ranging from 20% to 40% on the parasite density were obtained [Figure 8].

Precision and Accuracy

Readers obtained 100% precision and accuracy [**Table III**].

Reader's performance

- **Detailed sensitivity and specificity analysis and predictive validity indices**

All positive slides as well as the negative ones were correctly identified by the four (4) readers [**Table IV**].

- **Determination of sensitivity, specificity and indices of predictive values**

The readers gained 100% sensitivity, specificity and predictive values [**Table V**].

Discussion

This work is the beginning of a series of evaluations falling within the framework of the quality approach with a view to the accreditation of the LPM-HALD according to the ISO 15189 Standard. It consisted of the verification of the microscopic method for malaria diagnosis at the LPM- HALD with the aim of supporting the laboratory in this process.

During this study, the value of the agreement varied between slides. This could be explained by the ability of readers to easily identify *Plasmodium falciparum*. The only *Plasmodium ovale* (*Po*) identification slide has been subject of discordance. This could be explained by the fact that slide readers are much less familiar with the identification of *P. ovale* which represents less than 2% of the plasmodial species circulating in Senegal as reported by a study carried out in Senegal, in 2018, and as notified by the national guide for the biological diagnosis of malaria published in Senegal, in 2015 [6, 14].

However, the results of this study showed that four (4) out of six (6) PD evaluation slides obtained a concordance of less than 50%. This could be explained by the difficulty that technicians encounter in performing a PD. Indeed, this difficulty is even recognized by the WHO in its quality assurance manual where the threshold for PD is rather lower (40% and 50% for levels 2 and 1 respectively) compared to the threshold for identification (80% and 90% for levels 2 and 1 respectively). Thus, the WHO and the Senegalese NMCP have recommended a new method for quantifying malaria parasites, based on the mandatory taking into account of the presence of the nucleus, cytoplasm and/or vacuole of the parasite before identifying it, and count it for PD [5, 6].

Our results have also, through the scores produced, and verified the skill levels of slide readers. Indeed, the four (4) readers obtained, on species identification, a score greater than or equal to 0.8 compared to the reference and a score ranging from 20% to 40% on the parasite density. This therefore corresponds to level 2 of the WHO which has retained the lower thresholds of 40% for Level 2 and 0.5 for Level 1. This level of performance achieved by the LPM-HALD slide readers is considered as satisfactory according to the WHO Quality Assurance Manual [5].

These results also verified the performance of the readers of the slides which obtained 100% fidelity and accuracy. This performance was confirmed by the detailed analysis of sensitivity and specificity and predictive validity indices for which readers obtained 100% sensitivity, specificity and predictive values. This means that all the positive slides as well as the negative ones were correctly identified by all four (4) readers indicating their performance in detecting the presence or absence of *Plasmodium*. These results exactly correspond to those expected by WHO and NMCP-Senegal [5, 6].

Overall, the performance characteristics measured during this work showed that the LPM-HALD is in line for accreditation according to ISO 15189 standards in relation to the microscopic diagnosis of malaria.

Conclusion

This study contributed to confirm the good performance of malaria microscopy diagnosis at the LPM-HALD. Also it highlighted difficulties linked to the quantification of the parasite density, suggesting the need to strengthen the training and upgrading of microscopists. This confirmed the importance of setting up a quality management system in order to ensure a continuous improvement. And finally, it confirmed the essential role of an accessible slide bank which facilitate a continuous and regular internal and external quality control in the laboratory to comply with the requirements of ISO 15189 and the NMCP in Senegal

List of abbreviations

IRS	Indoor residual spray
AQ	Amodiaquin
SMC	Seasonal Malaria Chemoprophylaxis
ACT	Artemisinin-based combination therapy
PD	Parasite Density
EEQ	Evaluation Externe de la Qualité
FN	False negative
FP	False positive
TS	Thick smear
HALD	Hôpital Aristide Le Dantec
LPM	Laboratoire de Parasitologie et Mycologie
LLINs	Long-lasting insecticidal nets
WHO	World Health Organization
NMCP	National Malaria Control Programme
Se	Sensitivity
Sp	Specificity
SP	Sulfadoxine-Pyriméthamine
RDT	Rapide Diagnostic Test
IPTp	Intermittent preventive treatment in pregmant women
UCAD	Université Cheikh Anta Diop de Dakar
TN	True negatives
TP	True positives
NPV	Negative Predictive Values
PPV	Positive Predictive Values

Ethics approval and consent to participate

Not Applicable

Consent for publication:

Not Applicable

Availability of data and materials:

The data supporting the fndings of this article are included within the article.

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The authors declare that they have no competing interests

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MNG, ABD, MAD, ASB, and DN conceived and designed the study. MNG, ABD, and MAD carried out the experiments and collected data. MNG, ABD, and MAD analysed the data. MNG, ABD, MAD, YD, AMM, MD, AS, DZ, YDN, LN, MSY, BD, AS, AT, MT, NG, AF, BN, DS, AN, AK, MFN, JFG, FD, GD, IMN, EMB, OB, CN, PON, AG, MS, TN, KN, MCS, MN, ASB, and DN contributed to writing the manuscript. All authors read and approved the fnal manuscript.

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