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External Quality Assessment of Malaria Microscopy in Hawassa Health Facilities, Southern Ethiopia

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Abstract: Background: Misinterpretation of malaria microscopy results can lead to inappropriate case management of malaria. The objective of this study was to assess the quality of malaria microscopy among health facilities in Hawassa city. A cross-sectional study was conducted to assess the quality of malaria microscopy diagnosis in Hawassa city health facility laboratories from November 2012 to January 2013 in Southern Ethiopia. Validated panel malaria slides were distributed to health facilities accompanied with a questionnaire that assessed factors related to malaria microscopy improvement. Operational definitions for correct result and major and minor errors were outlined. A total of 51 laboratory professionals in 10 health facilities were surveyed with a response rate of 85%. Results were collected and data was analyzed by SPSS, and WinPepi software. Result: Of 306 malaria slides examined in Sample 1-Sample 6 [S1-S6] only 54% of the examinations reported correctly. Considering major errors in [S1-S4], the most common errors were reporting negative for positive slide 39/83(47%), species identification error 29/83(35%) and density 15/83 (18%). In mixed Plasmodium falciparum/Plasmodium vivax (Pf/Pv) sample, only 18% of participants made correct diagnosis in identifying both Pf/Pv species. In Plasmodium negative sample 45(88.2%) of participants scored (no parasites observed) correctly. Considering S1-S4, 29 of the 165 densities reported were different from the reference density established for each slide. 53% of participants had never participated in a formal training on malaria microscopy, and among those who did, more than half were trained earlier than 2008. All of the participants reported to use tap water in preparation of working Giemsa solution. Conclusion: The present assessment revealed a poor quality of malaria microscopy in Hawassa city administration health facilities. Therefore, responsible bodies are required to improve quality of malaria microscopy, and also provide regular refreshment training for laboratory professionals in malaria microscopy. Further similar study should be conducted in large scale.

Keywords: External Quality Assessment, Malaria Microscopy

1. Introduction

In Ethiopia, malaria is a serious public health problem. Unlike other sub-Saharan African countries, malaria is caused by two malaria parasite species in Ethiopia: Plasmodium falciparum and P. vivax, which account for 60 percent and 40 percent of cases, respectively (1). Microscopic diagnosis of malaria based on examination of blood films stained with Giemsa is the gold standard method of diagnosis. This method of diagnosis also gives quantitative results that can be used in the evaluation of the degree and rate of clearance of parasitaemia. Microscopy remains to be the mainstay of parasite-based diagnosis in most large health clinics and hospitals in Ethiopia (2).

Early diagnosis and prompt, effective treatment is the basis for the management of malaria and key to reducing malaria mortality and morbidity (3). Ethiopia’s treatment guidelines recommend artemether-lumefantrine as the first line treatment for P. falciparum and chloroquine for P. vivax malaria. In addition, since 2005, the country has changed its antimalarial drug policy from chloroquine and fencider as first line treatment of uncomplicated malaria to Coartem,
which is a more expensive treatment that requires definitive
diagnosis to confirm cases of malaria prior to treatment in
order to reduce unnecessary treatment. To ensure that patients
are appropriately treated with the correct drug, it is important
that patients receive quality parasitological diagnosis (2, 3, 4).

However, like all detection methods, microscopy is an
imperfect technique (5). Poor microscopy has long been
recognized in practice and is a function of multiple factors,
including training and skills maintenance, slide preparation
techniques, workload, condition of the microscope, and
quality of essential laboratory supplies. Even among local
laboratories with similar equipment and equal training and
among reputed experts, abilities vary significantly (6, 7).

To assure quality in malaria microscopy an effective
quality control (QC)/quality assurance (QA) system engaging
different organizational levels is needed. This involves
standardization of procedures and establishment of national-
level diagnostics centers responsible for developing training
modules, training, identifying the materials needed to support
microscopy QA, and improving the performance and
maintaining the competence of microscopists is essential (7,
8).

In the absence of accurate laboratory diagnosis false
negatives can result in untreated malaria patients and
potentially severe consequences, including death. False
negatives can also significantly undermine both clinical
confidence in laboratory results and credibility in the
community. False-positive results are equally problematic.
Patients presenting with fever not caused by malaria may be
misdiagnosed and the true cause of their fever not treated.
This can also have severe consequences, including the death
of the patient. In addition, misdiagnosis of malaria will result
in the unnecessary prescription of high cost drugs and the
unnecessary exposure of the patient to potentially toxic drugs.
This is a needless burden to both the patient and the medical
services (8, 9, 10).

The objective of this study therefore is to assess the quality
of malaria microscopy diagnosis and identify factors which
might help improve quality of malaria microscopy results to
reduce the occurrence of incorrect results (false positive and
false negative results).

2. Materials and Methods

A cross-sectional study was conducted to assess the quality
of malaria microscopy diagnosis in Hawassa city
health facility laboratories from November 2012 to January
2013 in Southern Nations Nationalities and Peoples Region
(SNNPR), Hawassa city. The city is located 275 kms south of
the capital Addis Ababa.

Data were collected by distributing a standardized, pre-
validated malaria panel slides and questionnaire for the study
populations. The malaria panel slides were prepared in sets of
6 slides of standardized malaria blood smears by
investigators according to WHO standard operating
procedures for establishing a national reference slide bank (3)
and then validated by three trained expert laboratory
professionals working at Hawassa Regional Laboratory.

The six malaria slides containing high density, low density,
mixed and negative malaria slides were included in a set
[Sample 1(Pf 3+) , Sample 2 (Pf1+), Sample 3 (Pv3+),
Sample 4 Pv (1+), Sample 5 mixes of Pf and Pv and Sample
6 Negative]. The blood films were made of thin and thick
preparation and stained with Giemsa.

The slides were distributed to the participant laboratories.
Laboratory professionals read all the six panel slides as if
they examined routine clinical blood film samples. After the
slide reading process was completed, the completed result
forms were submitted to the investigators. Each result was
exhaustively checked for completeness and accuracy. All
complete data was entered in to a computer and summarized
using Ms Excel. Statistical analysis was done using SPSS;
version 19, 95% confidence interval was calculated using
Win Pepi version 11.

Ethical clearance was obtained from Hawassa University.
Permission was also obtained from all study health facilities.
Informed written consent was obtained from individual blood
donors for slide preparation. In addition, informed verbal
consent was obtained from individual laboratory professionals prior to reading of panel slides. Personal
information was kept confidential. Only coding was used to
differentiate between results.

Operational definitions were outlined for the results
reported, as correct, major and minor errors. Identification of
correct parasite and density of parasitemia were grouped in
correct result. Major error included reporting negative for a
positive slide and vice versa, reporting non P. falciparum for
a P. falciparum slide or non P. vivax for a P. vivax slide,
density variation ≥ 2+ from the reference, and when no
species identification or no density is reported. Minor error
were categorized as reporting density variation of 1+ from the
reference, and reporting mixed infection for a single
species of malaria. For the Pf/Pv mixed sample, correct
diagnosis was considered as reporting both Pf and Pv,
reporting negative and single species of parasite were
considered as major and minor errors respectively. For the
negative sample, reporting positive regarded as a major error.
Error in density refers to correct parasite identification but
deviation in density report from the reference.

3. Result

3.1. Microscopy

This study reported the findings of an assessment of
malaria microscopy in 10 health facilities in Hawassa city
administration. The survey involved 51 laboratory
professionals from 8 health centers and 2 hospitals. 71% of
laboratory professionals were from health centers and 29%
from hospitals. The response rate was 85%. In the assessment
306 malaria slides [S1-S6] were distributed to the health
facilities laboratories.

Overall 54% (166/306) of the slides sent for examinations
turned with correct result with [48.5% -59.9%] 95 %
Confidence Interval, while 32% (99/306) and 14% (41/306) were reported with major and minor errors, with [27.1% - 37.9%] and [9.8% - 17.7%] 95% Confidence Interval, respectively. (Figure 1)

Among major errors in [S1-S4], the most common errors were reporting negative for positive slide 39/83 (47%), species identification error 29/83(35%) and density 15/83 (18%).

In Sample 1, (Pf-3+) slide (Table 1) 17 (33%) of the participants reported major error of which only 1 of participants reported ‘negative’ for Pf 3+ slide; 4 (8%) of them made minor error, while 30 (59%) participants reported correct results. In the low density Pf 1+ slide (Sample 2), 27 (53%) of participants reported correctly and 23 (45%) of participants made major error of which 14 responded ‘negative’, and the rest were reported with minor error (Table 2). In P. vivax 3+ slide, correct responses were obtained from 23 (45%) participants. Major and minor errors were made by 24 (47%) and 4 (8%) participants respectively (Table 3). In Sample 4, P. vivax 1+ slide, 18 (35%) and 1 (2%) of participants made major and minor errors respectively, and 32 (63%) of participants reported correctly (Table 4).

For mixed sample 5, 10 (20%) of participants made correct diagnosis in identifying both Pf/Pv species, 10 (20%) and 60% of participants made major and minor errors respectively. For Plasmodium negative (sample 6), 44 (86.2%) of participants scored (no parasites observed) correct. Major errors among the remaining included reporting of P. falciparum malaria 1(2%), P.vivax 3(6%) and mixed 3(5.8%).

| Parasite density (‘plus system’) | Total |
|----------------------------------|-------|
| Reported result | No parasite observed | 1+ | 2+ | 3+ | 4+ | N (%) |
| P. falciparum | 6* | 1 | 30** | 2 | 39 (76%) |
| P.falciparum and P.vivax | | 1 | 1(2%) |
| P.vivax | 8* | 2* | 10 (19.6%) |
| No parasites observed | 1* | | | | 1(2%) |
| Total | 1 | 6 | 1 | 38 | 5 | 51(100%) |

**Correct result; *Major error

Table 1. Results for sample 1: P.falciparum, parasite density ”+++”.

Figure 1. Pie chart showing percent results reported from all slides.

Figure 2. Type of major error in S1-S4.
Table 2. Results for sample 2: P. falciparum, parasite density ″+″.

| Parasite density ('plus system') | Reported result | 1+ | 2+ | 3+ | 4+ | N (%) |
|----------------------------------|-----------------|----|----|----|----|-------|
| P.falciparum                      | No parasite observed | 27** | 1 | 1* | | 29 (56.8%) |
| P.vivax and P.falciparum         |                 | 7* | 1* | | | 8 (15.7%) |
| No parasites observed             |                 | 14* |   |   | | 14 (27.4%) |
| Total                            |                 | 34 | 2 | 1 | | 51 (100%) |

**Correct result; *Major error

Table 3. Results for sample 3: P. vivax, parasite density ″+++″.

| Parasite density ('plus system') | Reported result | 1+ | 2+ | 3+ | 4+ | N (%) |
|----------------------------------|-----------------|----|----|----|----|-------|
| P.vivax                          | No parasite observed | 5* | 4 | 23** | | 31 (60.7%) |
| P.vivax and P.falciparum         |                 | 1* |   | | | 1 (2%) |
| P.falciparum                     |                 | 2* |   | | | 2 (4%) |
| No parasites observed             |                 | 16* |   | | | 17 (33.3%) |
| Total                            |                 | 6 | 6 | 23 | | 51 (100%) |

**Correct result; *Major error

Table 4. Results for sample 4: P. vivax, parasite density ″+″.

| Parasite density ('plus system') | Reported result | 1+ | 2+ | 3+ | 4+ | N (%) |
|----------------------------------|-----------------|----|----|----|----|-------|
| P. vivax                         | No parasite observed | 32** | 1 | 2* | | 35 (68.6%) |
| P.vivax and P.falciparum         |                 | 9* |   | | | 9 (17.6%) |
| P.falciparum                     |                 | 7* |   | | | 7 (13.3%) |
| No parasites observed             |                 | 7 |   | | | 7 (100%) |

**Correct result; *Major error

3.2. Density Reports

Considering S1-S4 (Table 1-4), 29 of the 165 densities reported were different from the reference density established for each slide [considering: deviation in density irrespective of the species]. The percentage of density error reported with a 1+ and ≥ 2+ deviations from the reference density are approximately equal to 7%. [Deviation in density while correct species identification]. This survey also indicated more difference in density reports in high density slides 23/165 (14%) than low density slides 6/165 (4%) from the reference.

3.3. Results from Questionnaire

Almost half of the participants have 2-6 years of experience in medical laboratory service (it is assumed they perform malaria microscopy regularly in endemic setting). A quarter of them have less than two years experience, and the rest quarters have more than six years of experience. Majority (73%) of the laboratory professionals possess a diploma in medical laboratory technology (MLT) training, while 10% have certificate (Junior Laboratory Technician) and 18% BSc degree in MLT. 53% of participants had never participated in a formal training on malaria microscopy, and among those who did, more than half were trained earlier than 2008. All of the participants purchase the stoke Giemsa solution and prepare the working Giemsa solution with tap water.

4. Discussion

Research showed that impaired microscopy based diagnosis in hospital laboratories is common. (12). Likewise, among 204 blood films examined from [S1-S4] 83 (41%) of them were reported with major error. Specifically 33% of these examinations had a false negative and species misidentification report. Errors in detection and identification of Pf 3+ and Pf 1+ slides were 22% and 43% respectively. Similarly an article in Congo reported 16% and 35% of participants reported a similar finding for Pf 4+ and Pf 1+ malaria, respectively (13). It is known that both P. falciparum and P.vivax infection are common in Ethiopia and species identification is essential for patient management (1). Although it is underreported, failure in identification of P.falciparum and P.vivax with routine microscopy is common (14). Our study revealed about 18% of participants failed to identify P. falciparum. Similarly the Congo article reported 23% of participants failed to identify Pf. A study in Ontario
Canada reported 27% of laboratories failed to correctly speciate Pf (15) and 21% (56/267) failure rate for Pf identification registered in London England (16). In P. vivax slides 11% of our participants failed to identify P vivax parasites. Overall, our survey revealed 1:3 of blood films sent for malaria microscopy examinations to Hawassa laboratories were incorrectly misinterpreted; a worse report than the 1:7 ratio in South Africa which was thought alarmingly high (17).

Researches indicated poor performance when blood films are from mixed infection and that underreporting of mixed-species infections is also common (12). In UK for example, (162/210) (77.1%) correct diagnosis were found from single infection slides while 1 out of 6 mixed infections (16.7%) correctly diagnosed (16). Study showed 13-27% correct identification in mixed slides while 76-92% correct result from single species identification (18). Our study found 18% correct species identification in mixed infection while slightly more than half correct diagnosis was found in single infection.

Moreover, this survey reported 6/51 (12%) of participants made false positive results in (Sample 6). The Congo survey reported about 20% false positive rate (13). In such considerable number of false positive results other diseases may be overlooked and not treated in a timely manner. This contributes to an increase in non-malaria morbidity and mortality, the misuse of antimalarial drugs, the development of parasite drug resistance, increased costs to the health services and patient dissatisfaction are the other consequences of false positive results (8,9,10)

4.1. Density

The chance of false negative results increases with decreasing parasite densities (7,19). Insufficient reading time, poor smear quality, lack of motivation, and poor equipment will also lead to false negative smears (20). Greater microscopist experience and increased examination time and number of microscopic fields examined reduce such an error (21,22). Likewise, this survey found more correct results in high density Pf 3+ slide (59%) than Pf 1+ slide (53%). Similarly, more major errors were obtained (45%) in Pf 1+ slide than Pf 3+ slide (33%). This phenomenon was also observed in the Congo survey where more correct results obtained in the high density (Pf 4+) sample than Pf 1+, and more major errors were obtained in the Pf 1+ slide than (Pf 4+) (14). When the density of slide is low, misidentification of parasite species and false negative report (reporting negative) is more likely than when the reference density is high according to our research. Reporting density of parasite is important in case management of malaria, therefore laboratory professionals should have the basic skills in calculating malaria parasitemia with standard techniques.

4.2. Questionnaire Results

Training: It has been found that half of the participants had never participated in a formal training on malaria microscopy after their formal years of training in College, and among those who did; more than half were trained earlier than 2008. This reflects there is scarcity of training and refreshment courses in malaria diagnosis. Giemsa quality: Errors in diagnosis are more common when Giemsa staining is poor (23). The slides used for the assessment in this survey were well prepared and stained with Giemsa. Therefore, the slide preparation and staining doesn’t have any relation with the poor microscopy results obtained in this survey. Additional data on interview of the preparation of Giemsa indicated all laboratories in Hawassa use tap water to prepare working Giemsa solution. Contrarily, in Congo, for preparation of the working solution, half (52.3%) of participants used a buffer solution, 29.9% and 17.8% used distilled water and regular water (mostly tap water) respectively. It is however recommended to use buffer solutions in preparation of working Giemsa to achieve optimal quality of staining in blood films (24).

Moreover, in an open ended interview question for improvement of malaria diagnosis in general and malaria microscopy in particular, majority of participants suggested the need of training and refreshment courses in regular manner. Secondly the requirement of External Quality Assessment was mentioned. Availability of quality of Giemsa solution and regular supply of methanol for thin film preparation were mentioned in relation to improvement of species identification in malaria diagnosis. Moreover, availability of good quality microscope and posting SOP (standard operating procedures) were also mentioned as necessity for the improvement of malaria diagnosis.

5. Conclusion

The present assessment revealed a poor quality of malaria microscopy in Hawassa city administration health facilities. The questionnaire interview indicated half of the laboratory professionals had no any formal training on malaria microscopy, and that the buffer used for preparation of Giemsa working solution is not appropriate. Therefore, this research recommends responsible bodies to help improve the quality of malaria microscopy and the knowledge and skill of laboratory professionals in malaria parasite examination. A limitation relates to the coverage of this assessment, as less than 8.3% of hospitals and 1% of the health centers in region wide were included. Therefore, other similar study should be conducted in large scale.

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Authors Contribution

BZ, GA, TG, EK, GB: proposal development, data collection and panel slide preparation.
References

[1] Tulu NA: Malaria. In The Ecology of Health and Disease in Ethiopia. 2nd edition. Edited by Kloos H, Zein AZ. Boulder, USA: Westview Press Inc; 1993:341–352.

[2] The Federal Democratic Republic of Ethiopia MOH. Malaria Diagnosis and Treatment Guidelines for Health Workers in Ethiopia 2nd Edition Addis Ababa July 2004

[3] World Health Organization: Malaria Microscopy Quality Assurance Manual, version 1. 2009.

[4] World Health Organization: Basic Malaria Microscopy - Part II. Tutor's Guide. 2nd edition. 2010.

[5] O'Meara WP, Barcus M, Wongsrichanalai C, Muth S, Jason D Maguire JD, Robert G Jordan RG, Prescott WR, McKenzie FE. Reader technique as a source of variability in determining malaria parasite density by microscopy Malaria Journal 2006, 5:118

[6] Durrheim DN, Becker PJ, Billinghurst K, Brink A. Diagnostic disagreement – the lessons learnt from malaria diagnosis inMpumalanga. S Afr Med J. 1997; 87:609–611.

[7] Maguire JD, Lederman ER, Barcus MJ, O'Meara WA, Jordon RG, Duong S, Muth S, Sismadi P, Bangs MJ, Prescott WR, Baird JK, Wongsrichanalai C. Production and validation of durable, high quality standardized malaria microscopy slides for teaching, testing and quality assurance during an era of declining diagnostic proficiency. Malar J. 2006; 5:92.

[8] World Health Organization. Malaria Light Microscopy. Creating a Culture of Quality. Report of WHO SEARO/ WPRO workshop on quality assurance for malaria microscopy, 18–21 April 2005; Kuala Lumpur, Malaysia. 2005.

[9] World Health Organization: Manual of basic techniques for a health laboratory. 2nd edition 2003.

[10] Ohrt C, Purnomo, Sutamihardja MA, Tang D, Kain K. Impact of microscopy error on estimates of protective efficacy in malaria-prevention trials. J Infect Dis. 2002; 186:540–546.

[11] SNNPRS RHB/Malaria Consortium: Implementation Guideline for Malaria Microscopy Diagnosis Quality Assurance. Hawassa, Ethiopia. September 2008.

[12] Johnston SP, Pieniazek NJ, Xayavong MV, Slemenda SB, Wilkins PP, da Silva AJ. PCR as a confirmatory technique for laboratory diagnosis of malaria. J Clin Microbiol. 2006; 44:1087–1089.

[13] Mukadi P, Gillet P, Lukuaka A, Atua B, Kahodi S, Lokombe J Muyembe JJ, Jacobs J. External quality assessment of malaria Microscopy in the Democratic Republic of the Congo. Malaria Journal 2011; 10:308

[14] McKenzie FE, Sirichaisinthop J, Miller RS, Gasser RA Jr, Wongsrichanalai C. Dependence of malaria detection and species diagnosis by microscopy on parasite density. Am J Trop Med Hyg. 2003; 69:372–376.

[15] Thomson S, Lohmann RC, Crawford L, Dubash R, Richardson H. External quality assessment in the examination of blood films for malarial parasite within Ontario, Canada. Arch Pathol Lab Med. 2000; 124:57–60.

[16] Milne LM, Kyi MS, Chiodini PL, Warhurst DC. Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. J Clin Pathol. 1994; 47:740–742.

[17] Dini L, Frean J: Quality assessment of malaria laboratory diagnosis in South Africa. Trans R Soc Trop Med Hyg 2003, 97:675–677.

[18] Kettell hut M M , Chiodini PL, Edwards H, Moody A. External quality assessment schemes raise standards: evidence from the UKNEQAS parasitology subschemes. Clin Pathol 2003; 56:927–932.

[19] McKenzie FE, Sirichaisinthop J, Miller RS, Gasser RA Jr, Wongsrichanalai C. Dependence of malaria detection and species diagnosis by microscopy on parasite density. Am J Trop Med Hyg. 2003; 69:372–376.

[20] Ohrt C, Obare P, Nanakorn A, Adhiambo C, Awoondo K, O'Meara WP, Remich S, Martin K, Cook E, Chretien JP, Lucas C, Osoga J, McEvoy P, Owaga ML, Odera JS, Ogutu B. Establishing a malaria diagnostics centre of excellence in Kisumu, Kenya Malaria Journal 2007, 6:79

[21] Dowling MA, Shute GT. A comparative study of thick and thin blood films in the diagnosis of scanty malaria parasitaemia. Bull World Health Organ. 1966; 34: 249–267.

[22] Trape JF. Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. Trans R Soc Trop Med Hyg. 1985; 79: 181–184.

[23] Payne D. Use and limitations of light microscopy for diagnosing malaria at the primary health care level. Bulletin of the World Health Organization, 1988; 66 (5): 621–626

[24] World Health Organization: Basic Malaria Microscopy - Part I. Learner’s Guide. 2nd edition. 2010.