

# Performance of Malaria Microscopy External Quality Assessment and networking among Health Facilities in West Amhara Region, Ethiopia

Banchamlak Tegegne (✉ [Banteg92@gmail.com](mailto:Banteg92@gmail.com))

Amhara Public Health Institute <https://orcid.org/0000-0002-9916-3162>

**Kefale Ejigu**

Amhara National Regional Health Bureau

**Getaneh Alemu**

Bahir Dar University

**Yeshimebet Fetene**

Amhara National Regional Health Bureau

**Kindye Endaylalu**

Amhara National Regional Health Bureau

**Mulatu Melese**

Amhara National Regional Health Bureau

---

## Research

**Keywords:** external quality assurance, re-checking, test agreement

**Posted Date:** January 31st, 2020

**DOI:** <https://doi.org/10.21203/rs.2.22333/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at BMC Infectious Diseases on May 19th, 2020. See the published version at <https://doi.org/10.1186/s12879-020-05077-5>.

# Abstract

**Background:** In most health facilities, microscopic examination of peripheral blood smears is performed for definitive diagnosis of malaria. Despite it produces reliable results about both the infection status and level of parasitemia, microscopic examination of malaria is affected by skill of the laboratory personnel, workload, condition of microscopes and quality of laboratory supplies. Therefore, continuous monitoring of the performance of laboratories is critical in order to make timely corrections.

**Objective:** To assess malaria microscopy performance of diagnostic laboratories in west Amhara region.

**Methods:** A facility based cross-sectional study was conducted from July 2017 to July 2019 among thirty malaria diagnostic laboratories in west Amhara region. Thirty slides were collected from participating laboratories every quarter. Collected slides were taken to Amhara Public Health Institute (APHI) reference laboratory and re-checked by malaria microscopist who were blind to the results from health facilities. Percentage of test agreement, rates of false positive, false negative and species misdiagnosis were calculated using Excel 2010.

**Results:** Among a total of 6689 slides re-examined, results of 6146 slides were the same with that of participating laboratories to give a test agreement of 97.31% and 94.6% in parasite detection and species identification, respectively. Variations in the overall performance of individual laboratories were seen within a range of 81.55% to 97.27% test agreement. Results of 543 (8.12%) slides were discordant, of which 363 (5.4%), 93 (1.4%) and 87 (1.3%) were due to species misdiagnosis, false positive and false negative results, respectively.

**Conclusion:** There was good test agreement between participated laboratories and APHI laboratory. More accurate performance is expected as the country is tracking to malaria elimination. Hence, strengthening the EQA program by integrating rechecking with onsite evaluation is recommended.K

## Background

Malaria is a febrile disease caused by intracellular haemoparasites of the genus plasmodium [1]. Despite tremendous efforts have been made to combat malaria, the disease still remains a global public health problem. In 2018 alone, about 228 million cases were reported globally with 405 thousand deaths. About 67% of the deaths occur among under-5 children, the most vulnerable group [1]. The burden outweighs in the tropics and subtropics that the World Health Organization (WHO) African region contributes for 93% of the cases and 94% of the deaths [1]. Based on the current stratification in Ethiopia, 60% of the population lives in risk areas; altitude and rain fall being important indicators [2]. About 1.5 million confirmed cases and 356 deaths were reported in 2017 in the country. Besides, *P. falciparum* (the most pathogenic species) is more prevalent in the country infecting 69% of confirmed cases in the same year [3]. Moreover, *Anopheles arabiansis*, a species responsible for malaria epidemics, is the primary vector transmitting malaria in Ethiopia leaving the country with a threat of outbreaks [1, 4].

Early diagnosis and treatment of cases helps to avoid complications and death due to malaria. It also decreases parasite transmission, misuse of anti-malaria drugs and development of drug resistance by parasites. Definitive diagnosis based on clinical manifestations is not possible because many of the signs and symptoms overlap with that of other febrile illnesses [5, 6]. Accordingly, based on the Ethiopian malaria national strategic plan, 100% of suspected cases should be diagnosed in the laboratory within 24 hours of fever onset [2].

Laboratory diagnosis of malaria is made by rapid diagnostic tests, blood film microscopy or molecular techniques [7]. In health facilities equipped with clinical laboratory, microscopic examination of stained thin and thick peripheral blood smears is the most commonly practiced technique. Because the technique is easily accessible and affordable in peripheral laboratories. It also produces reliable results about both the infection status and parasitemia level [8]. Microscopy has a sensitivity of detecting as few as 5–10 parasites/ $\mu$ l of blood [9]. However, microscopic examination of malaria is affected by multiple factors including skill of the laboratory workforce, workload, condition of microscopes and quality of laboratory supplies [9]. Hence, it is of primary concern to ensure diagnostic services: which provide accurate results; are administered by competent and motivated staff supported by effective training, supervision and quality control. Diagnostic laboratories should also be supported by a logistics system to provide and maintain adequate supplies of reagents and equipment [6].

Assessment of the diagnostic performance could be made by involving laboratories in External Quality Assurance (EQA) program [9]. It is a vital tool for identifying and assessing technology in use, identifying gaps in laboratory performance and targeting training needs. It can be performed through panel testing, blind re-checking and/or onsite evaluation [10]. In Ethiopia, the regional central laboratories are mandated to perform EQA in health facilities of respective regions. Hence, Amhara Public Health Institute (APHI) engages health facilities located in the region. Compiled data on EQA performance of involving laboratories helps to inform common problems and recommend for corrective actions as well as to monitor the impact of EQA on malaria microscopy performance of health laboratories. A similar study has been conducted in west Amhara before five years; however the results might not be consistent as there is difference in laboratory staff (due to turnover and recruitment), training, patient flow, quality of supplies and test procedures. Therefore, the aim of the present study was to show the recent two years malaria microscopy performance of health facility laboratories in west Amhara region as assessed through blind rechecking.

## Methods

### Study Design and Area

A facility based cross-sectional study was conducted from July 2017 to July 2019 among thirty malaria diagnostic health facilities in west Amhara region. The region has seven zones and two city administrations [9]. There are 39 hospitals, 523 health centers and one public health institute (APHI) in the region. Amhara Public Health Institute directly conducts EQA for malaria diagnosis in selected

hospital and health center laboratories in the region. Selection of laboratories is based on availability and scope of human resource, strength of internal quality control and EQA performance, commitment of the health facility and laboratory management to perform EQA, training on laboratory quality management and safety, geographical proximity for supporting peripheral laboratories and proficiency of the laboratory from previous EQA participation. Those health facility laboratories, in turn, serve as 'EQA centers' for peripheral health laboratories under their cluster. Hence, all health laboratories in Amhara region are networked to APHI malaria EQA program (Figure 1). Head quarter of the institute is located at Bahir Dar city and has one branch at Dessie. Both the head quarter and Dessie branch perform similar EQA activities. The Bahir Dar site performs EQA among health facilities in the western Amhara region and the Dessie site recruits health facilities from eastern region. In the present study, thirty facilities in the western region were recruited. These thirty health facilities were addressed by blind rechecking program quarterly (every 3 months).

## **Study Procedure**

Thirty health facility laboratories (24 hospitals and 6 health centers) participated in the EQA program for malaria diagnosis performed by Bahir Dar site of APHI and all of them were included in the present study. The malaria diagnosis performance was assessed through blind rechecking of stained blood film slides collected from laboratories and onsite supervision. Participating laboratories were requested to store both positive and negative slides. Then, malaria laboratory experts from APHI went to the sites at every quarter (3 months) and collected 30 slides following the WHO recommendation [9]. Selection of slides was made from the registration log book just to avoid bias of selecting slides based on the smearing and staining quality. Collected slides were taken to APHI reference laboratory and rechecked by malaria microscopists who were blind to the results from health facilities. Discrepant results were re-examined again by a senior quality officer and his/her finding was taken as the final result. After each round, malaria laboratory experts from APHI visited participating laboratories to show errors of discordant slides and to give practical training so that the performance of laboratories will be improved. The institute has also sent a written feedback to all participating laboratories. The feedback contains information about discordant slides, smearing and staining quality of slides and gaps to be improved. Discordant management form was also administered to laboratory professionals where discordant results were reported. The form contains list of possible reasons for inaccurate microscopy results. In this way, EQA data collected for 8 consecutive quarters from July 2017 to July 2019 were included for the present study. Data were entered and analyzed in Microsoft Excel 2010. Percentage of agreement as well as rates of false positive, false negative and species misdiagnosis were calculated.

## **Results**

A total of 25 health facility laboratories (19 hospitals and 6 health centers) participated in all the 8 rounds of EQA. Three hospital laboratories participated in 7 rounds and two hospital laboratories participated in 6 and 5 rounds each. In order to maximize representativeness of the data, we have included data from all 30 health facilities collected between July 2017 and July 2019. A total of 6689 slides were collected and

re-examined by malaria laboratory experts in APHI. Results of 6146 slides were the same with that of participating laboratories to give an overall result agreement of 91.88%. Test agreements in parasite detection and species identification were 97.31% and 94.6%, respectively. Results of 543 (8.12%) slides were found to be discordant. Variations in the performance of individual laboratories were seen within a range of 81.55% to 97.27% result agreement (Table 1). Analysis of performance by EQA round revealed the lowest (88.73%) and the highest (96.30%) test agreements in round 3 and 5, respectively. Performance of laboratories didn't show uniform trend in every round of EQA (Table 2). More than half of the discordant results (363 out of 543) were due to species misdiagnosis while the rest 93 and 87 slides were false positive and false negative results, respectively. The highest frequency of species misdiagnosis was seen in slides positive for mixed infection that 91 slides with mixed infection were reported as *P. vivax* (Table 3).

## Discussion

Early diagnosis of malaria plays an important role not only for prompt treatment of patients but also for malaria prevention and control. Ethiopia has set a malaria control strategic plan to be implemented from 2017 to 2020. Goals of the plan include reducing malaria cases by 40% (the baseline being 2016 data), maintain near zero deaths and implement malaria elimination in 239 districts by 2020. Laboratory diagnosis of all cases within 24 hours of fever onset in 2017 and beyond was one of the strategic objectives to achieve the goals [4]. For accurate case detection and successful malaria elimination, quality of diagnosis is indispensable. Despite blood film microscopy is the gold standard technique, it is prone to errors both in the smear preparation and staining as well as parasite detection, species identification and quantification. Therefore, periodic in service trainings are given to laboratory personnel and their performance is monitored through EQA programs by experts from central laboratories.

The ultimate goal of the EQA program is to enhance the quality of malaria diagnosis by improving the competency of laboratory personnel and quality of laboratory utilities [11]. Therefore, all health facility laboratories should be benefitted by participating in the program. However, implementing EQA directly managed at national or regional centers is too costly in terms of time, logistics and human power. This brings difficulty in sustained implementation of the program especially in resource limited countries like Ethiopia. Considering this, APHI has decentralized the malaria EQA program since 2012 (Fig. 1). We believe other regions or countries will be benefitted if they adopt the decentralized and networked EQA implementation approach.

Despite it was planned that 30 slides were to be collected from each laboratory, less number or no slides were collected in some rounds (Table 1). This was due to political instability in areas where respective health laboratories are located. The mean test agreement in detecting malaria parasites in the present study (97.31%) was in line with previous results from Amhara region of Ethiopia (96.6%) [12], and Pakistan (99.0-99.5%) [13]. On the other side the test agreement was higher than recent results of 78%, 88% and 91.7% from Oromia region of Ethiopia [14], Hawassa [15] and Addis Ababa [16], respectively. Variations in laboratory workload, training and assessment methods might bring the difference. Periodic

in service training given to laboratory personnel accompanied with close supervision and feedback after each round of EQA is thought to bring the high accuracy in detecting malaria parasites in the region.

Among 180 (2.69%) discordant slides in parasite detection, 87 (1.4%) were false positive results which was similar with previous findings of 2%, 2.64% and 4.05% from Canada [17], west Amhara region [12] and Addis Ababa [16], respectively. On the contrary, higher false positive rates of 7.8%, 24.6% and 24.4% were reported from USA [18], Congo [19] and Ethiopia [14], respectively. Similarly, frequency of false negative reporting was also low (1.3%) in the present study, implying that the overall performance of health facilities in malaria parasite detection is acceptably good. However, as the country is moving from malaria control to elimination, any non-zero report of false positive and/or false negative will be significant [4]. Among interviewed laboratory professionals, 25% responded that they observe less than 100 fields before reporting negative slides due to high workload. This contributes for false negative results.

Treatment of malaria varies according to the infecting plasmodium species [20]. Therefore, laboratories should identify and report species correctly. The proportion of species mis-diagnosis in the present study (5.4%) goes in line with previous results of 3.4% in the same study area [12] and it is much lower than previous studies from Hawassa [15] and Oromia [14] where the laboratory professionals correctly identified the species in 74.3% and 44.6% of malaria positive slides during panel testing, respectively. The discrepancy might be due to difference in the method of assessment and the status of EQA and other supportive activities from reference laboratories. In general, different factors encountering at the pre-analytical, analytical and post-analytical steps of malaria microscopy equally contribute for discordant results. In the present study, correct reporting of *P. falciparum*, *P. vivax* and mixed infection was a major problem identified. Failure to prepare and identify species from thin film might be a possible reason as 25% of interviewed professionals responded that they identify species from thick blood film. Similarly 20% and 10% of professionals reported gap in training and experience, respectively. Quality of smearing and staining also contribute for correct parasite detection and species identification, which were not assessed due to inconsistency of such data.

## Conclusion

There was good agreement in parasite detection (97.31%) and species identification (94.6%) between peripheral laboratories and malaria experts in APHI in west Amhara region. However, there was no regular trend of improvement in overall performance across the eight rounds of EQA. More accurate malaria microscopy performance is also expected as the country is tracking to malaria elimination. Hence, ensuring optimum workload and strengthening the EQA program by integrating rechecking with onsite evaluation is recommended.

## Abbreviations

APHI      Amhara Public Health Institute

EQA External Quality Assurance

WHO World Health Organization

## Declarations

### Ethics Approval and Consent to Participate

The study was ethically approved by APHI institutional review board and official permission was obtained from all participating health facilities. Data from each facility was presented anonymously using code. All discordant results were also communicated to respective laboratories.

### Consent for Publication

Not applicable.

### Availability of Data and Materials

The original data for this study is available from the corresponding author.

### Competing Interests

The authors declare that they have no competing interests.

### Funding

No fund was obtained for execution of the study.

### Authors' Contributions

BT conceived the project. BT and GA designed the project. BT, KE, GA, YF, KE and MM performed the experiment and analyzed the data. BA and GA wrote the manuscript. All authors read and approved the final manuscript.

### Acknowledgements

We thank Amhara national regional state health bureau for giving ethical approval. We are also grateful for the management team and laboratory personnel working in all targeted laboratories for giving permission and participation in the EQA activities.

## References

1. World Health Organization. World malaria report 2019.

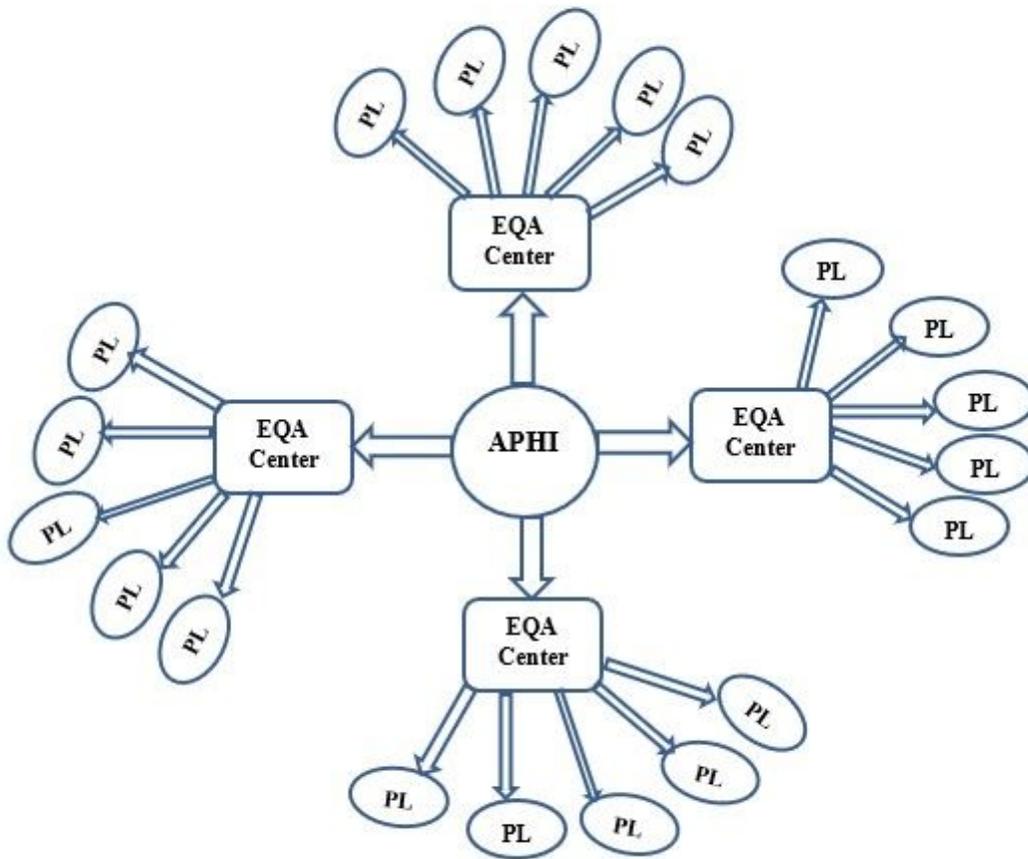
2. Federal Ministry of Health. National Malaria Strategic plan 2014-2020. 2014;10-11.
3. World Health Organization. World malaria report 2017.
4. President's malaria initiative Ethiopia. Malaria operational plan FY 2019.
5. Raghuveer C, Rajeev A, Bhandari P. Comparative study of peripheral blood smear, quantitative buffy coat and modified centrifuged blood smear in malaria diagnosis. *Indian J Pathol Microbiol.* 2008; 51:108-112.
6. Ethiopian Health and Nutrition Research Institute. Manual for the laboratory diagnosis of malaria, First Edition. Federal Ministry of Health. 2012.
7. Cheesbrough M. District Laboratory Practice in Tropical Countries, part 1, 2<sup>nd</sup> edition, Cambridge University Press, New York, USA, 2009: 454.
8. Fantahun B, Yeshambel B, Jemal A, Fisha W, Nigussu G, Afework K, *et al.* Does the practice of blood film microscopy for detection and quantification of malaria parasites in northwest Ethiopia fit the standard? *BMC Health Serv Res.* 2014; 14: 529.
9. World Health Organization. Malaria Microscopy Quality Assurance Manual – Version 2. 2016.
10. Jane Y carter. External Quality Assessment in Resource-limited countries. *Biochemia Medica.* 2017; 27(1): 97-109.
11. World Health Organization. Malaria Microscopy Quality Assurance Manual – Version 1. 2009.
12. Hiwot A, Melasu B, Leykun D, Mulatu M, Zelalem D, Manamnot A, *et al.* External quality assessment of malaria microscopy diagnosis among public health facilities in west Amhara Region, Ethiopia. *BMC Research Notes.* 2017; 10(764): <https://doi.org/10.1186/s13104-017-3080-0>.
13. Muhammad A, John D, Muhammad A, Muhammad A, Nayyar G, Zarfishan T. District level external quality assurance (EQA) of malaria microscopy in Pakistan: pilot implementation and feasibility. *Malaria Journal.* 2011; 10(45): <http://www.malariajournal.com/content/10/1/45>.
14. Getachew S, Olifan Z, Geletta T, Abdi S. External quality assessment of malaria microscopy diagnosis in selected health facilities in western Oromia, Ethiopia. *Malaria Journal.* 2018; 17(233): <https://doi.org/10.1186/s12936-018-2386-2>.
15. Ayalew F, Tilahun B, Taye B. Performance evaluation of laboratory professionals on malaria microscopy in Hawassa Town, Southern Ethiopia. *BMC Research Notes.* 2014; 7(839). DOI:[10.1186/1756-0500-7-839](https://doi.org/10.1186/1756-0500-7-839)
16. Leykun D, Honelgn N. Assessment of Malaria Microscopic Diagnosis Performance of Laboratory Professionals in Addis Ababa's Public Health Facilities. *Biomedical Sciences.* 2019; 5(1):1-6. doi: 10.11648/j.bs.20190501.11
17. Thomson S, Lohmann RC, Crawford L, Dubash R, Richardson H. External quality assessment in the examination of blood films for malarial parasites within Ontario, Canada. *Arch Pathol Lab Med.* 2000; 124: 57-60.
18. Edson ED, Glick T, Massey L. Detection and identification of malaria parasite: a review of proficiency test results and laboratory practice. *Lab Med.* 2010; 41(12): 719-23.

19. Pierre M, Philippe G, Albert L, Ben A, Simelo K, Jean L, *et al.* External quality assessment of malaria microscopy in the Democratic Republic of the Congo. *Malaria Journal*. 2011; 10(308): <http://www.malariajournal.com/content/10/1/308>.
20. Federal Ministry of Health. National malaria guideline, third edition. 2012.

## Tables

Due to technical limitations, tables are only available as a download in the supplemental files section

## Figures



EQA center; health facility laboratories directly participated in the APHI EQA program and included in the present study, PL; peripheral health facility laboratories

**Figure 2**

Amhara Public Health Institute regional EQA networking for malaria microscopy

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables.docx](#)
- [Tables.docx](#)