External quality assessment of malaria microscopy diagnosis in selected health facilities in Western Oromia, Ethiopia

Getachew Sori, Olifan Zewdie, Geletta Tadele and Abdi Samuel

Abstract

Background: Accurate early diagnosis and prompt treatment are one of the key strategies to control and prevent malaria disease. External quality assessment is the most effective method for evaluation of the quality of malaria microscopy diagnosis. The aim of this study was to assess the quality of malaria microscopy diagnosis and its associated factors in selected public health facility laboratories in East Wollega Zone, Western Ethiopia.

Methods: Facility-based cross-sectional study design was conducted in 30 randomly selected public health facility laboratories from November 2014 to January 2015 in East Wollega Zone, Western Ethiopia. Ten validated stained malaria panel slides with known Plasmodium species, developmental stage and parasite density were distributed. Data were captured; cleaned and analyzed using SPSS version 20 statistical software-multivariate logistic regressions and the agreement in reading between the peripheral diagnostic centers and the reference laboratory were done using kappa statistics.

Results: A total of 30 health facility laboratories were involved in the study and the overall quality of malaria microscopy diagnosis was poor (62.3%). The associated predictors of quality in this diagnosis were in-service training (AOR = 16, 95% CI (1.3, 1.96)), smearing quality (AOR = 24, 95% CI (1.8, 3.13)), staining quality (AOR = 15, 95% CI (2.35, 8.61)), parasite detection (AOR = 9, 95% CI (1.1, 8.52)) and identification skills (AOR = 8.6, 95% CI (1.21, 1.63)). Eighteen (60%) of health facility laboratories had in-service trained laboratory professionals on malaria microscopy diagnosis.

Conclusion: Overall quality of malaria microscopy diagnosis was poor and a significant gap in this service was observed that could impact on its diagnostic services.

Keywords: External quality assessment, Malaria microscopy, Western Oromia
and other concentration techniques, such as quantitative
buffy coat (QBC) method, rapid diagnostic tests (RDTs)
and molecular diagnostic methods [2].

The World Health Organization (WHO) recommends
cross-checking of blood slides. A sample of routine
blood slides is sent to the reference laboratory, where it is
checked for accuracy. External quality assessment (EQA)
programmes is an alternative approach. In such pro-
grammes, the reference laboratory sends stained blood
film samples to the peripheral laboratories, which assess
them and submit a report, after which they are given
feedback about the correct results and their own perfor-
mance [4].

The high sensitivity of diagnosis in malaria-endemic
areas is particularly important for the most vulnerable
population groups, such as young children and non-
immune populations, in whom the disease can rapidly
be fatal [5]. Malaria control requires a functional labo-
atory set-up with quality diagnostic service, trained
professionals and microscopists to halt the burden. This
work requires concentration in order to assess the qual-
ity of blood film malaria microscopy for the detection of
Plasmodium species by proficient testing, blinded slide
rechecking using checklist to identify any gaps in provid-
ing malaria services in selected health facility laborato-
ries in the Western Oromia, Ethiopia.

Methods

Study area and period
This study was conducted in East Wollega Zone of the
Oromia National Regional State, Western Oromia,
Ethiopia, from November 2014 to January 2015. The
temperature was 10.9–33.9 °C, annual rainfall was 1000–
2400 mm and topography was 4.91% high land, 53.17%
mid land, and 41.92% low land. The zone has 61 public
health facilities, one of which is a referral hospital, one
da district hospital, and 59 health centres [6]. All health
facility laboratories provide malaria microscopy services,
except Gaba Jimata health centre in Gida Ayana Woreda.
The study was conducted in 30 health facility laboratories
that were randomly selected from 60 malaria microscopy
providing public health facilities.

Study procedures
Verified stained panel slides were distributed to 30 labo-
atory professionals from selected public health facili-
ties and the results were collected. The validated panel
slides were distributed for reading to all selected malaria
microscopy health facility laboratory professionals. The
panel slides included 10 stained samples with different
composition of Plasmodium species, the developmen-
tal stage of the parasite and parasite density as recom-
manded by national guidelines [7]. The time allowed for
reading 10 slides was 50–70 min according to national
guidelines recommend examination of 100/HPF (High
Power Field) (100 × objective). EQA of malaria micros-
copy is an essential requirement for malaria care in a
district. The focus of EQA is on the identification of labo-
ratories where there may be serious problems resulting
in poor performance, not on identification of individual
slide errors or validation of individual patient diagnosis.
It helps to ensure the trust-worthiness of smear results
through the following:

On-site evaluation
Malaria laboratory activities in all selected malaria
microscopy diagnosis in the health facilities were
observed and all heads of departments of the respective
health facilities were interviewed using WHO-AFRO
checklists to obtain a realistic assessment of the overall
operational conditions and skills in the laboratory.

Blind rechecking
Slides are rechecked for quality of blood film preparation,
staining and accuracy of result. Rechecking reflects the
true performance of routine diagnostic services at health
facility level. For this study, the blood film slides were col-
lected from the selected malaria microscopy providing
health facilities, which were selected in accordance with
WHO recommendation of a minimum of five positives
and five negatives slides per month.

Data analysis
Data were captured, cleaned and analysed using SPSS
version 20 statistical software-multivariate logistic
regressions and P value of less than 0.05 was considered
to be statistically significant. The specificities, sensitivi-
ties, positive predictive value, negative predictive value
of slide reading by the laboratory professionals were
assessed. Agreement in reading between peripheral diag-
nostic centres and the reference laboratory readings were
interpreted using kappa value. Accuracy is defined as the
closeness of the measured result to the true value and
malaria microscopy was used as the gold standard in the
study.

Results

Quality of malaria microscopy: panel slides
A total of 300 panel slides were distributed to 30 malaria
microscopy diagnosing centres for 30 laboratory person-
nel. Of the total facilities, 6 (20%) of laboratory profes-
sionals scored an excellent agreement with reference
reader (kappa = 1.00) on parasite detection and 6 (20%)
scored slight agreement (kappa = 0.0–0.2) (Table 1).

Based on national guideline for evaluation of labora-
tory professionals on panel slide examination, 1 (3.3%) of
laboratory professionals correctly read all positive slides with correct parasite quantification. Twelve (40%) did not try to report parasite density. 17 (56.7%) correctly quantified the parasite density in at least one positive slide which agreed with the reference density established for each slide. Six (20%) of laboratory professionals reported all positive slides as positive and 20 (66.7%) correctly reported all negative slides. Twenty-nine (96.7%) of participants missed species identification in at least one positive slide (Table 2).

Of 300 panel slides, 240 positive panel slides were distributed which comprised 90 (37.5%) *Plasmodium falciparum*, 90 (37.5%) *Plasmodium vivax* and 60 (25%) mixed of *P. falciparum* and *P. vivax*. Forty-two (46.7%) and 47 (52.2%) of the slides were correctly detected and identified for *P. falciparum* and *P. vivax*, respectively. Detection error was reported in 33 (36.7%) for *P. falciparum*, 22 (24.5%) for *P. vivax* and 70% *Plasmodium* species identification error from mixed infection. Health facilities that participated in the EQA programme had considerable agreement (kappa = 0.75) with reference reader on malaria detection by microscopy when compared with health facilities that did not participate in the EQA programme (kappa value = 0.31). Comparison between in-service training in malaria detection was higher in trained laboratory professionals (kappa = 0.58) when it was compared with untrained in-service professionals in selected health facilities (kappa = 0.56) (Table 3).

### Table 1 Sensitivity, specificity and agreement of each health facility laboratory professionals with level 1 malaria microscopist on malaria microscopy diagnosis Western Oromia, Ethiopia

| Id of HF lab. | Sensitivity % | Specificity % | NPV | PPV | Agreement (%) | Kappa value |
|---------------|---------------|---------------|-----|-----|---------------|-------------|
| Lab 1         | 88            | 50            | 50  | 88  | 80            | 0.4         |
| Lab 2         | 71            | 50            | 20  | 100 | 70            | 0.4         |
| Lab 3         | 88            | 50            | 20  | 88  | 80            | 0.6         |
| Lab 4         | 75            | 100           | 50  | 100 | 80            | 0.5         |
| Lab 5         | 83            | 50            | 25  | 100 | 80            | 0.7         |
| Lab 6         | 88            | 100           | 67  | 100 | 90            | 0.7         |
| Lab 7         | 88            | 100           | 67  | 100 | 90            | 0.7         |
| Lab 8         | 100           | 100           | 100 | 100 | 100           | 1.0         |
| Lab 9         | 63            | 100           | 40  | 100 | 70            | 0.4         |
| Lab 10        | 63            | 50            | 25  | 83  | 60            | 0.1         |
| Lab 11        | 88            | 100           | 67  | 100 | 90            | 0.7         |
| Lab 12        | 71            | 100           | 50  | 83  | 80            | 0.6         |
| Lab 13        | 100           | 100           | 100 | 100 | 100           | 1.0         |
| Lab 14        | 75            | 100           | 50  | 100 | 80            | 0.5         |
| Lab 15        | 100           | 100           | 100 | 100 | 100           | 1.0         |
| Lab 16        | 63            | 50            | 25  | 83  | 60            | 0.1         |
| Lab 17        | 88            | 100           | 67  | 100 | 90            | 0.7         |
| Lab 18        | 75            | 100           | 50  | 100 | 80            | 0.5         |
| Lab 19        | 63            | 50            | 25  | 83  | 60            | 0.1         |
| Lab 20        | 88            | 100           | 67  | 100 | 90            | 0.7         |
| Lab 21        | 100           | 100           | 100 | 100 | 100           | 1.0         |
| Lab 22        | 63            | 100           | 40  | 100 | 70            | 0.4         |
| Lab 23        | 33            | 50            | 20  | 80  | 40            | 0.3         |
| Lab 24        | 63            | 100           | 40  | 100 | 70            | 0.4         |
| Lab 25        | 63            | 50            | 25  | 83  | 60            | 0.1         |
| Lab 26        | 75            | 100           | 50  | 100 | 80            | 0.5         |
| Lab 27        | 88            | 100           | 67  | 100 | 90            | 0.7         |
| Lab 28        | 50            | 50            | 20  | 80  | 50            | 0.0         |
| Lab 29        | 100           | 100           | 100 | 100 | 100           | 1.0         |
| Lab 30        | 100           | 100           | 100 | 100 | 100           | 1.0         |
| 77%           | 83.3%         |               | 78% | 0.5 |               |             |
Random blind rechecking

Overall sensitivity and specificity of health facilities in detection and identification of Plasmodium species were 78 and 83.7%, respectively. The overall false positive and false negative rates were 98 (24.4%) and 85 (14.4%), respectively and the overall agreement between health facility laboratory and regional laboratory experts on malaria microscopy diagnosis (random blind rechecking) was 82% (kappa = 0.62).

Professional background and number of laboratory professionals in selected laboratories

The selected health facility laboratories had a total of 53 laboratory professionals, of which 17 (32%) were degree and 36 (68%) were diploma level educated. 17 (56.7%) of health facilities had 2 laboratory professionals and 12 (40%) 1 laboratory professional. Of the laboratory professionals, 39 (73.6%) were trained in malaria

| HF Lab. ID | Positive reported as negative or vice versa (zero points/slide) | Positive reported as positive (three points/slide) | Correct species (three points/slide) | Correct parasite stage (two points/slide) | Correct parasite load (two points/slide) | Negative reported as negative (ten points per slide) | Cumulative score | Performance |
|------------|----------------------------------------------------------------|--------------------------------------------------|-------------------------------------|----------------------------------------|----------------------------------------|------------------------------------------|----------------|------------|
| Lab-1      | 2*0 = 0                                                        | 21                                               | 12                                  | 8                                      | 8                                      | 10                                         | 59             | Poor       |
| Lab-2      | 3*0 = 0                                                        | 15                                               | 6                                   | 4                                      | 6                                      | 20                                         | 51             | Poor       |
| Lab-3      | 5 x 0 = 0                                                      | 12                                               | 0                                   | 0                                      | 0                                      | 10                                         | 22             | Poor       |
| Lab-4      | 2*0 = 0                                                        | 18                                               | 9                                   | 6                                      | 4                                      | 20                                         | 57             | Poor       |
| Lab-5      | 4 x 0 = 0                                                      | 15                                               | 6                                   | 4                                      | 0                                      | 10                                         | 35             | Poor       |
| Lab-6      | 1 x 0 = 0                                                      | 21                                               | 18                                  | 12                                     | 4                                      | 10                                         | 65             | Poor       |
| Lab-7      | 1 x 0 = 0                                                      | 21                                               | 8                                   | 8                                      | 4                                      | 20                                         | 61             | Poor       |
| Lab-8      | No error                                                       | 24                                               | 24                                  | 16                                     | 16                                     | 20                                         | 100            | Excellent  |
| Lab-9      | 3 x 0 = 0                                                      | 15                                               | 0                                   | 0                                      | 0                                      | 20                                         | 35             | Poor       |
| Lab-10     | 4 x 0 = 0                                                      | 15                                               | 0                                   | 0                                      | 0                                      | 10                                         | 25             | Poor       |
| Lab-11     | 1 x 0 = 0                                                      | 21                                               | 21                                  | 14                                     | 6                                      | 20                                         | 82             | Good       |
| Lab-12     | 2 x 0 = 0                                                      | 18                                               | 12                                  | 8                                      | 6                                      | 20                                         | 64             | Poor       |
| Lab-13     | No error                                                       | 24                                               | 18                                  | 12                                     | 6                                      | 20                                         | 80             | Good       |
| Lab-14     | 2 x 0 = 0                                                      | 18                                               | 3                                   | 2                                      | 0                                      | 20                                         | 43             | Poor       |
| Lab-15     | No error                                                       | 24                                               | 21                                  | 14                                     | 6                                      | 20                                         | 85             | Good       |
| Lab-16     | 4 x 0 = 0                                                      | 15                                               | 3                                   | 2                                      | 0                                      | 10                                         | 30             | Poor       |
| Lab-17     | 1 x 0 = 0                                                      | 21                                               | 18                                  | 12                                     | 8                                      | 20                                         | 79             | Good       |
| Lab-18     | 2 x 0 = 0                                                      | 18                                               | 9                                   | 6                                      | 4                                      | 20                                         | 57             | Poor       |
| Lab-19     | 4 x 0 = 0                                                      | 15                                               | 6                                   | 4                                      | 0                                      | 10                                         | 35             | Poor       |
| Lab-20     | 1 x 0 = 0                                                      | 21                                               | 18                                  | 12                                     | 6                                      | 20                                         | 77             | Good       |
| Lab-21     | No error                                                       | 24                                               | 21                                  | 14                                     | 4                                      | 20                                         | 83             | Good       |
| Lab-22     | 3 x 0 = 0                                                      | 15                                               | 3                                   | 2                                      | 0                                      | 20                                         | 40             | Poor       |
| Lab-23     | 5 x 0 = 0                                                      | 12                                               | 6                                   | 4                                      | 0                                      | 10                                         | 32             | Poor       |
| Lab-24     | 3 x 0 = 0                                                      | 15                                               | 3                                   | 2                                      | 0                                      | 20                                         | 40             | Poor       |
| Lab-25     | 4 x 0 = 0                                                      | 15                                               | 9                                   | 6                                      | 2                                      | 10                                         | 42             | Poor       |
| Lab-26     | 2 x 0 = 0                                                      | 18                                               | 9                                   | 6                                      | 0                                      | 20                                         | 53             | Poor       |
| Lab-27     | 1 x 0 = 0                                                      | 21                                               | 15                                  | 10                                     | 6                                      | 20                                         | 72             | Poor       |
| Lab-28     | 5 x 0 = 0                                                      | 12                                               | 0                                   | 0                                      | 0                                      | 10                                         | 22             | Poor       |
| Lab-29     | No error                                                       | 24                                               | 21                                  | 14                                     | 8                                      | 20                                         | 87             | Good       |
| Lab-30     | No error                                                       | 24                                               | 21                                  | 14                                     | 8                                      | 20                                         | 87             | Good       |
| Overall average points | 57 | Poor |
microscopy diagnosis. Fifty (94.3%) of the laboratory personnel had service of 2 and more years.

Factors associated with the quality of malaria microscopy
To refine any confounding factors, a multivariate logistic regression model was used. According to this model, factors such as in-service training, quality of staining and quality of smearing, remained the predictors for quality of malaria microscopy. Trained laboratory professionals on malaria microscopy diagnosis and quality assurance were 16 times more likely to produce quality of malaria microscopy diagnosis than untrained laboratory professionals [(AOR = 16, 95% CI of (1.3–1.96)]. Health facility laboratories preparing good stained blood films were 10 times more likely to harvest good quality in the malaria microscopy diagnosis than poorly staining blood films [(AOR = 15, 95% CI of (2.35, 8.61)]. Preparing good blood films was 24 times more likely in quality of malaria microscopy than poorly performing blood films [(AOR = 24, 95% CI of (1.8, 3.13)] (Table 4).

Discussion
The overall quality of malaria microscopy in the assessed public health facility laboratories was 62.3%, which was considered to be poor. An ISO 15189 document requirement for quality and competence recommends above or equal to 80% [8]. This difference may be due to lack of training in malaria diagnosis and quality assurance, but was similar to the study conducted in Pakistan in which quality of malaria microscopy diagnosis was poor [9].

The current study revealed, 18 (60%) of health facility laboratories had in service trained laboratory professionals on malaria microscopy and a better quality of malaria microscopy diagnosis than those with no trained laboratory professionals [(AOR = 16 (1.3–1.96)]. A similar study conducted in health facilities in Oromia Regional State indicated 24% of health facilities participated laboratories

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**Table 3** Overall sensitivity, specificity and agreement of public health facility laboratory professionals with level 1 malaria microscopist in detecting malaria parasites Western Oromia, Ethiopia

| Peripheral laboratory | Sensitivity | Specificity | Agreement % | Kappa value |
|-----------------------|-------------|-------------|-------------|-------------|
| In-service training   |             |             |             |             |
| Trained               | 81.2        | 90.9        | 83.2        | 0.578       |
| Untrained             | 64.0        | 68.7        | 65          | 0.56        |
| EQA participation      |             |             |             |             |
| Participated          | 89.4        | 96.1        | 90.7        | 0.746       |
| Not participated      | 66.9        | 76.4        | 68.8        | 0.3077      |
| Qualification         |             |             |             |             |
| B.Sc. degree          | 82.5        | 90          | 84          | 0.592       |
| Diploma               | 73.8        | 82.5        | 75.5        | 0.42        |

**Table 4** Factors associated with quality of malaria microscopy in selected public health facility laboratories Western Oromia, Ethiopia

| Variables                | Malaria microscopy quality | OR (95%) CI | P-value |
|--------------------------|----------------------------|-------------|---------|
|                          | Yes (%)                   | No (%)      | COR     | AOR   | |
| EQA participation        | 8 (80%)                   | 2 (20%)     | 22 (3.1–163) | 10 (0.4–2231) | 0.561 |
| Use of buffered water    | 7 (77.8%)                 | 2 (22.2%)   | 19 (27–145) | 0.1 (0.012–1.07) | 0.44 |
| Internal quality control | 4 (19%)                   | 17 (81%)    | 1.00     | 1.00  | |
| Practice staining quality| 5 (71.4%)                 | 2 (28.6%)   | 7 (1.07–14.6) | 15 (2.35–18.6) | 0.039* |
| In service training      | 6 (26.1%)                 | 17 (73.9%)  | 1.00     | 1.00  | |
| Qualification            |                           |             |          |       |       |
| Diploma                  | 6 (30%)                   | 14 (70%)    | 2 (0.48–11) | 0.4 (0.09–2.05) | 0.285 |
| B.Sc.                    | 5 (30%)                   | 5 (50%)     | 1.00     |       |       |
| Smearing quality         | 7 (70%)                   | 3 (30%)     | 0.1 (0.19–0.61) | 24 (1.8–31.3) | 0.037* |
| No                       | 4 (20%)                   | 16 (80%)    | 1.00     | 1.00  | |

1—reference group
COR crude odds ratio, AOR adjusted odds ratio, CI confidence interval

* Significant at P-value < 0.05
in malaria microscopy diagnosis [10] while in Ethiopia 7 (6%) of health facilities participated in malaria microscopy diagnosis [11]. According to malaria laboratory diagnosis EQA scheme guidelines, laboratory professionals must have adequate training on malaria microscopy diagnosis and quality assurance to maintain quality implementation [7]. The study conducted in Hawassa health facility showed 50% of health facilities had trained laboratory professionals more than not trained [12]. This reflects a scarcity of training and refresher courses in malaria microscopy diagnosis. Low sensitivity and specificity on malaria parasites diagnosis indicated that there were many false negative results; which can lead to delayed treatment, development of serious complications and death.

This study showed that 80% and above of collected slides were good in staining in 7 (23.3%) of the health facility laboratories and had better quality in malaria microscopy diagnosis than health facility laboratories with poor blood film staining qualities. The difference was statistically significant ([AOR = 15, 95% CI (2.35, 8.61)] which was slightly better than 20% in the Democratic Republic of the Congo [13], but less than health facilities in Ethiopia at 31 (47%) [11].

Smearing and staining quality in the study area were known to be poor in routine laboratory settings, which has a great impact on patient results. Poor blood film preparation and staining generates artifacts commonly mistaken for malaria parasites, including bacteria, fungi, stain precipitation, dirty and cell debris. Normal blood components, such as platelets, also confound diagnosis. Improved training and higher quality of smear preparation and staining are required to reduce false readings.

The number of health facility laboratories with good detection and identification of Plasmodium species was 15 (50%) and 20 (66.7%), respectively. But the overall agreements of health facility laboratory professionals on detection and identification of plasmodium species with reference reader were 78 and 44.6% which was less than the national guideline recommendation [7]. It was also less than the study conducted in Africa 82% in parasite identification [14]. However, similar to detection with the study conducted in North Gondar (77%) [15].

Because of economic constraints, we did not assess all health facilities that perform malaria microscopic examination. Moreover, due to time limitation, the study could not evaluate the performance of health facilities regarding the quality of blood film preparation and staining procedures.

**Conclusion**

In all assessed health facilities, malaria laboratory diagnosis was available but the overall quality of malaria microscopy diagnosis was poor. A significant gap was observed which could significantly impact on malaria microscopy quality services including untrained laboratory professionals on malaria microscopy diagnosis and quality assurance, poor blood film preparation, poor staining quality, poor parasite detection and identification.

**Abbreviations**

QBC: quantitative buffy coat; WHO: World Health Organization; EQA: external quality assessment; ISO: International Standard Organization.

**Authors’ contributions**

GS was the primary researcher, conceived the study, designed, participated in sample collection, performed laboratory experiments, conducted data analysis and drafted the manuscript for publication. OZ, AS and GT participated in the interpretation of the results and reviewed the initial and final manuscript. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The data and material set supporting the results of this article is included within the article.

**Consent for publication**

Not applicable in this section.

**Ethics approval and consent to participate**

Ethical permission was obtained from the Ethical and review committee of Wollega university. Written informed consent was obtained from each participant before collection of samples.

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