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Performance Assessment of Laboratory Professionals on Malaria Smear Microscopy and Associated Factors in West Oromia Malaria External Quality Assessment Centers, Ethiopia 2025

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Abstract

Background

Ensuring competency among laboratory personnel is crucial for accurate malaria microscopy. Accurate laboratory diagnosis is essential for effective treatment, reducing drug resistance, and guiding proper care. Yet, with diagnostic responsibilities now shifted to general laboratory technicians, concerns have emerged about their proficiency, especially in regions like West Oromia. Misdiagnosis and poor diagnostic quality of malaria microscopy in the area.

Methods

A cross-sectional study design was conducted among 131 laboratory professionals from 25 external quality assessment centers in West Oromia between January and March 2025. Demographic data were collected using a self-administered questionnaire. Each professional was assessed using 10 pre-validated malaria slide panels focusing on malaria parasite detection, species differentiation, and parasite quantification. Data were entered into EpiData and analyzed using SPSS version 20. Descriptive statistics summarized the findings, and diagnostic performance metrics were calculated. Bivariate analysis ($p < 0.25$) identified candidates for multivariate logistic regression, where $p < 0.05$ indicated statistical significance.

Results

The acceptable proficiency test result was 62.6%, with high sensitivity (95.7%) and specificity (96.2%), with strong overall agreement (95%) and a high kappa value ($k = 0.91$). Agreement in the identification of different malaria species was 69%, with a kappa value of 0.52, reflecting moderate concordance. Parasite quantification by laboratory professionals was very low, with only 3.4% counting parasites within the expected range. Multivariate analysis revealed that having a diploma/Level 4 education (AOR = 4.87; 95% CI: 1.5–15.6; $p < 0.05$) and professionals who had not received training within the past three years (AOR = 27.3; 95% CI: 8.1–91.6; $p < 0.001$) were significantly associated with the competency of laboratory professionals in diagnosing malaria smear microscopy.

Conclusion

There were high unacceptable proficiency test results among laboratory professionals; high parasite detection agreements, moderate species identification, and poor parasite counts were observed. The performance status of laboratory professionals in diagnosing malaria smear microscopy was associated with educational levels and recent malaria training. Training and promoting higher education

among laboratory staff were important to enhance the diagnostic accuracy of malaria microscopy in West Oromia.

1. BACKGROUND

Malaria is a life-threatening disease caused by the infection of red blood cells with protozoan parasites of the genus *Plasmodium*, transmitted to humans through the bites of infected female Anopheles mosquitoes. Five species of Plasmodium (*P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi*) most commonly infect humans (1). Of all the malaria species, *Plasmodium vivax* is the most common, causes severe, even fatal infections, and contributes significantly to global morbidity and mortality, although *Plasmodium falciparum* is the cause of more deaths (2). A fifth species, *P. knowlesi* (a Plasmodium species that mainly infects non-human primates), is increasingly reported in people living in forested regions of some countries (3).

Malaria transmission is influenced by several factors that contribute to environmental, behavioral, and biological factors, including the presence of *Anopheles* mosquito breeding sites (stagnant water), climate conditions (temperature, rainfall, humidity), lack of vector control measures (insecticide-treated nets, indoor residual spraying), low immunity (children under 5, pregnant women, non-immune travelers), and socioeconomic factors (poor housing, limited healthcare access) (4). Microscopic examination of Giemsa-stained blood films remains the gold standard for malaria diagnosis due to its ability to detect and quantify parasites and differentiate species.

Malaria remains a leading cause of morbidity and mortality worldwide, with one million deaths occurring annually (16). In Ethiopia, despite its dramatic decline over the last two decades, malaria remains one of the biggest problems for public health and socio-economic development. In the last ten years, the number of malaria cases has reportedly decreased from 3.8 million to around 1.2 million in 2021; the number of deaths also decreased from 261 in 2010 to 132 in 2021. However, the District Health Information Software 2 (DHIS2) report for 2020 shows that the number of malaria cases in Ethiopia increased by 34 percent compared to 2019 data (the number of confirmed malaria cases in 2019 was 904,405, while in 2020 it was 1,389,750). Triangulation of the data with previous Public Health Emergency Management (PHEM) and malaria microplanning reports shows that more than 80 percent of the malaria burden in Ethiopia is among adults and children aged five years and older (17).

High-quality malaria diagnosis is crucial for improving patient care, as accurate detection helps ensure proper treatment, reduces unnecessary use of antimalarial drugs, and enhances malaria surveillance. Misdiagnosis can lead to harmful consequences, such as the prescription of expensive or toxic drugs, potentially causing drug resistance. In addition, false-negative results can delay treatment for infected patients, leading to severe complications or death, especially in vulnerable populations. Correct diagnosis also aids in identifying non-malaria cases, ensuring that patients receive the appropriate care (9; 18).

Delayed diagnosis and inadequate treatment, particularly for children under five, pregnant women, and individuals with low immunity, can cause uncomplicated malaria to progress quickly into severe or complicated forms, significantly increasing mortality risk. The primary complications associated with severe malaria are the leading causes of death, particularly among young children. Therefore, timely and accurate diagnosis is essential to prevent the progression of the disease, guide appropriate treatment, and minimize the risk of fatal outcomes in high-risk groups (19).

In Ethiopia, malaria diagnosis in laboratories was previously done by malaria microscopists at malaria control offices. Following decentralization and program integration, general laboratory technicians now handle malaria diagnostic services at health facilities. However, the proficiency of these technicians in detecting and identifying malaria is *a* paucity of reports in Ethiopia, especially in the study area (20). Misdiagnosis, which leads to mistreatment, is a great challenge in reporting accurate and reliable results in malaria microscopy diagnosis in Ethiopia (21).

Sustaining good laboratory practice is one of the greatest challenges to building quality laboratory systems to deliver accurate, reliable, and timely results in our country, and the overall quality of malaria microscopy diagnosis in western Oromia was 62.3% (22). Therefore, the purpose of this study was to evaluate the effectiveness of malaria smear microscopy diagnostics and the variables influencing their quality in West Oromia hospitals that provide services.

2. METHODS

Study Area and Period

The study was conducted at 25 Malaria EQA center hospitals in the western parts of Oromia, which includes 8 zones and 6 city administrations, namely West Shoa, H/G/Wallaga, East Wallaga, West Wallaga, Kelam Wallaga, Buno Bedele, I/A/Bora, and Jimma Zone, and 6 (Ambo, Nekemte, Jimma, Agaro, Nedjo, and Mattu). Western parts of the Oromia region consist of 45 public hospitals, 527 public health centers, and one public health research and referral laboratory center providing health care services for the community. All hospitals and health centers widely provide different healthcare and diagnostic services, including malaria microscopy diagnosis. This study was conducted from January to March 2025.

Study Design

A hospital-based cross-sectional study design was undertaken to assess the performance of laboratory professionals in diagnosing malaria smear microscopy and assessment of associated factors.

Population

Source Population

The source population of this study consisted of 260 laboratory professionals working at 25 EQA Centers of governmental hospitals in western Oromia.

Study Population

A simple random sample was selected of 131 laboratory professionals working at 25 EQA Centers of governmental hospitals in western Oromia.

Eligibility Criteria

Inclusion Criteria

All laboratory professionals working at West Oromia EQA Centers, performing malaria smear microscopy, and being present in the study health facility during data collection were included.

Exclusion Criteria

Laboratory personnel absent from work during data collection were excluded from the study.

Sample Size Determination and Procedure

Sample Size Determination

The sample size was determined using the standard formula: n = [z² p(1-p)] / e² / 1 + [z² p(1-p)] / e² * N, and a 95% confidence level, 0.22 (35) The malaria microscopy percentage of agreement was 78%, as previously reported in West Oromia (22). with a marginal error of 5%. Thus, a sample size of 131 will be calculated. Population size = 260

Sample size formula n = [z² p(1-p)] / e² / 1 + [z² p(1-p)] / e² * N]

Z = 1.96 (95% confidence level)

N = 260 Population size

P = 0.78 (78%) from the previous article's standard deviation

Q = 0.22 p + q = 1 q-1 = p

E = 0.05 (5% margin of error)

Sample size n = 3.84 * 0.17 / 0.0025 / 1 + 3.84 * 0.17 / 0.0025 * 260 = 261.12 / 2 = 130.5

The sample size is 131.

Sampling Procedure

A simple random selection of the study population, microscopists from the selected hospitals.

Data Collection Procedure

Data were collected by distributing a standardized, pre-validated malaria slide panel and self-administered questionnaires. Proficiency panel slides for malaria microscopy are made up of ten characterized slides, five positive and five negatives (36). Panel slides from the national malaria slide bank were prepared as smears and stained by EPHI according to WHO. Panel slide preparation and this slide Validated by WHO. Level 1 Certified Lab personnel at the Nekemte Public Health Research and Referral Laboratory (NPHRRL). The data collector and microscopes are obtained from NPHRRL, and the data is collected with the support of the NPHRR and PI by coordinating visits to the malaria EQA center health facility for various malaria quality-related activities. These ten-panel slides were administered to study participants for the performance assessment on parasite detection and parasite load count with the WBC method in thick blood film for quantification and species identification.

Ten minutes per slide were allocated to each participant to examine the blood film slides (9). At the same time, self-administered questionnaires were utilized to collect sociodemographic characteristics and factors associated with the performance of laboratory professionals on malaria smear microscopy.

Data Quality Assurance

Orientation was provided for data collectors by the principal investigator on how to distribute validated slides and questionnaires. The questionnaire and slides were pretested before actual data collection was conducted at Wallaga University Referral Hospitals. After the questionnaire was complete and the slides were examined, data obtained from the questionnaire and results from the slide reading were checked for completeness and accuracy by the principal investigator.

Study Variables

Dependent Variable

- Malaria smear microscopy diagnosis status.

Independent Variable

- Age
- Sex
- Sensitivity
- Specificity
- Percent of agreement
- Species Identification
- Quantification
- Educational level
- Training status on malaria diagnosis

- Work experience
- Type of Binocular Microscope

Operational Definition

Acceptable

Achieves a PT score of 80% or more.

Unacceptable

Fails to reach the PT score of < 80%.

Excellent

≥ 90% correct identification of malaria species, stages, and parasite density.

Very Good

80–< 90% correct identification of malaria species, stages, and parasite density.

Good

70–< 80% correct identification of malaria species, stages, and parasite density.

Poor

<70% correct identification of malaria species, stages, and parasite density (9).

Data Analysis Technique

Score Interpretation for Malaria PT Slide Results (36).

Score Per Slide	*Definition*	
	Correct	***Incorrect***
10	Parasite species identification Parasite stage identification	
10	Negative slide report correctly	
8	Parasite species identification Parasite stage identification	*Parasite load Parasite*
8	Parasite species identification Parasite Load	*Parasite stage identification*
6	Parasite species identification	Stage identification Parasite load
5	Parasite load	Parasite species identification Stage identification
5	Parasite stage identification	Parasite species identification Parasite load
0		Positive report as negative or vice versa

Scoring on each of the 10 panel slides was worth 10 points. For parasite load count with the WBC method in thick blood film, variation up to ± 25% of the mean is acceptable (36).

After the completeness and consistency of data were checked by the principal investigator, data was entered & cleaned by Epidata and analyzed using SPSS for Windows version 20. Descriptive statistics were used to summarize the data and evaluate the performance of the malaria microscopy diagnostic laboratory. Sensitivity, specificity, false positive rate, false negative rate, misdiagnosis rate, parasite load, and percentage of agreement will be calculated and presented by graphs and tables. Variables from bivariate regression with P-value < 0.25 candidate variables to multivariate logistic regression. Finally, variables from multivariate logistic regression with a p-value < 0.05 were identified as factors affecting the performance of malaria laboratory diagnosis. The odds ratio with a 95% confidence interval was calculated to determine the strength of the association. The percentage agreement in readings between the participants and expert microscopists, malaria microscopists was classified into four based on WHO recommendations: “Poor” (< 70% agreement), “Good” (70%—<80%), “Very good” (80%—<90%), and “Excellent” (> 90%). Those categories were summarized into two for Excellent and Very Good (> 80%). Acceptable for the category “Good,” and for “Poor” (< 80%), unacceptable (20). The Kappa value was calculated to see the strength of the agreement.

Ethical Consideration

A letter of ethical clearance was obtained from the Research Ethics Committee of Wallaga University, Institute of Health Sciences. The official letter was written with the reference number (IHSREC006/052/2025). Consent was obtained from all the study participants after providing brief information about the objectives and the aim of the research.

3. RESULTS

Sociodemographic Characteristics of Study Participants

The majority of the study participants were less than 30 years old, male, and hold a BSc & above and more than 2 years of work experience, and they had trained in malaria microscopy within the past 3 years. Most of the study hospitals use Olympus brand microcopy and used both thick and thin films for malaria diagnosis. Most of the laboratory personnel did not perform parasite counts in the routine malaria diagnosis (Table 1).

Table 1
Socio-demographic characteristics of laboratory professionals who participated in the performance evaluation of malaria smear microscopy in west Oromia Malaria EQA center 2025.

Variables	Category	Number (n = 131)	Percent (%)
Age	> 41 Years	8	6.1
	31–40	24	18.3
	< 30 Years	99	75.6
Sex	Male	92	70.2
	Female	39	29.8
Educational level	BSc and above	96	73.3
	Diploma/Level 4	35	26.7
Work Experience	> 2 Years	72	55
	< 2 Years	59	45
Malaria Training	Trained < 3 Years ago	62	47.3
	Trained > 3 Years ago	25	19.1
	Not Trained	44	33.6
Microscope Functionality	Yes	131	100
	No	0	0
Type of Microscope Used	Olympus	72	55
	Primo Star	32	24.4
	CX	23	17.6
	Other	4	3.1
Malaria Diagnosis Method used	Thin Film only	1	0.8
	Thick Film Only	6	4.6
	Thick and Thin Film	124	94.7
Parasite Count perform	Yes	37	28.2
	No	94	71.8
Parasite count Method	+, ++, +++	25	67.6
	Parasite per microliter/WBC	12	32.4
	Parasite per micro/RBC	0	0

Variables	Category	Number (n = 131)	Percent (%)
	Percent of RBC infected	0	0

Quality Assurance and Diagnostic Practices in Malaria Microscopy

The majority of study hospital laboratories had available SOPs and EQA (External Quality Assessment) guidelines, and all laboratory professionals participated in EQA. Most of laboratories participate in all types of EQA methods, including onsite evaluations, rechecking blind readings (RBR), and proficiency testing (PT). Specifically, 96.9% conduct quality control for those performing QC, with 73.2% doing this regularly. Additionally, 73.3% use buffered water to prepare stains, while 98.5% scan at least 100 fields before reporting a slide as negative. Furthermore, all laboratories involved in the study reported positive cases, including species identification and parasite stage (Table 2).

Table 2
Quality Assurance and diagnostic practice on malaria microscopy related variables.

Variables	Category	Number (n = 131)	Percent (%)
Available SOP	Yes	127	96.9
	No	4	3.1
Availability of EQA guideline	Yes	127	96.9
	No	4	3.1
EQA participation	Yes	131	100
	No	0	0
Type of EQA Participated	Onsite Evaluation	2	1.5
	RBR	9	6.9
	PT	5	3.8
	All	115	87.8
Quality control performance	Yes	127	96.9
	No	4	3.1
QC performing	Regularly	93	73.2
	Not Regularly	34	26.8
Staining Reagent	Write Stain	4	3.1
	Giemsa Stain	127	96.9
Use of buffered water to prepare Giemsa stain working reagent	Yes	96	73.3
	No	35	26.7
Minimum field scan to report negative	=/>100 field	129	98.5
	< 100 field	2	1.5
Report of positive results by species identification	Yes	131	100
	No	0	0
Report of the result by stage identification	Yes	131	100
	No	0	0

Diagnostic Test Performances

The study evaluated the performance of a diagnostic test by comparing its results against a reference standard of 10 panel slides assessed by 131 study participants. The malaria diagnosis performance of laboratory professionals on the ten slides showed high sensitivity (95.7%) and specificity (96.2%), with very strong overall agreement (95%) and a kappa value of 0.91 (Table 3).

Table 3
Diagnosis performance of laboratory professionals on malaria smear microscopyin west Oromia Malaria EQA center 2025.

	Expected Slide Result						
Participant Results	Positive	Negative	Total	Sensitivity	Specificity	Agreement	Kappa
Positive	630(95.7%) TP	25(3.8%) FP	655	95.7	96.2	0.95	.91
Negative	28(4.3%) FN	627(96.2%) TN	655				
Total	658	652	1310				

Species Identification Performance

Readings of the five malaria-positive blood film slides were evaluated to assess participants' ability to correctly identify parasite species (*Plasmodium falciparum* (*P. f.), Plasmodium vivax (P. v.)*, and mixed infections). Out of the BF slides expected to be *P. falciparum*, 195 (74.4%) were correctly identified as *P. falciparum* by the participants. However, 48 slides (18.3%) were misclassified as mixed infections, and 19 slides (7.3%) were misidentified as *P. vivax*; for BF slides expected to be *P. vivax*, 202 (77.1%) were correctly identified. Misclassifications included 45 slides (17.2%) as mixed infections and 15 slides (5.7%) as *P. falciparum*. Among the BF slides expected to be mixed infections, only 56 (42.7%) were correctly identified. misdiagnosed as *P. falciparum* 48 slides (36.6%) and as *P. vivax* 27 slides (20.7%). A significant proportion of mixed infections were misdiagnosed as either *P. falciparum or P. vivax*. The overall agreement between participant and expected results was low at 69%, with a Kappa statistic of 0.52, indicating moderate agreement (Table 4).

Table 4
Species identification performance of laboratory professionals on malaria smear microscopy in west Oromia Malaria EQA center 2025.

	Expected Slide Result				Agreement	Kappa
Participant Results	P. F	P. V	Mixed	Total		
P. F	195 (74.4%)	15 (5.7%)	48 (36.6%)	258 (100%)	0.69	.52
P. V	19 (7.3%)	202 (77.1%)	27 (20.7%)	248 (100%)		
Mixed	48 (18.3%)	45 (17.2%)	56 (42.7%)	149 (100%)		
Total	262 (100%)	262 (100%)	131 (100%)	655 (100%)		

Performance of WHO PT result category, final PT result status, and parasite density

The majority of participants, 54.2%, demonstrated "very good" performance, and 34.4% showed “good” performance. Additionally, 96.6% of participants reported parasite densities that were "out of range" (Fig. 1).

The final overall PT result status was 62.6% acceptable (Fig. 2).

Factors associated with the performance status of laboratory professionals in diagnosing malaria smear microscopy

This study assessed the factors associated with the performance status of laboratory professionals in diagnosing malaria using smear microscopy. The multivariate analysis identified several significant factors that influenced competency, including educational level and malaria-related training. Professionals with a bachelor’s degree (BSc) or higher demonstrated significantly better competency compared to those with diplomas or Level IV qualifications ($p < 0.01$). In the multivariate analysis, diploma holders were 4.87 times more likely (AOR = 4.87, 95% CI: 1.5–15.6%, $p < 0.05$) to have unacceptable performance. Training on malaria diagnosis was one of the predictors of malaria smear microscopy performance. Professionals who had not received any training were 27.3 times more likely to fall into the unaccepted competency group compared to those who had been trained within the past three years (AOR = 27.3; 95% CI: 8.1–91.6%, $p < 0.001$) (Table 5).

Table 5
factors associated with competency status among participants

Variables	Categories	Competency Status		Bivariate		Multivariate	
		Accepted (%)	Unaccepted (%)	P-Value	COR (95%CI	P-Value	AOR (95%CI
Age	< 30Years	58(70.7%)	41(83.7%)	1			
	31–40 Years	18(22%)	6(12.2%)	.14	0.47		
	> 41 Years	6(7.3%)	2(4.1%)	.37	.47		
Sex	Male	61(74.4%)	31(63.3%)	1			
	Female	21(25.6%)	18(36.7%)	.18	1.68		
Education	BSc and above	71(86.6%)	25(51%)	1		1	
	Dip/Level4	11(13.4%)	24(49%)	.00	6.19	0.00	4.87(1.5,15.6) *
Experience	> 2 Years	57(69.5%)	15(30.6%)	1			
	< 2 Years	25(30.5%)	34(69.4%)	.00	5.16		
Training	Trained < 3	54(65.9%)	8(16.3%)	1		1	
	Trained > 3	20(24.4%)	5(10.2%)	.4	1.6	.19	2.48(0.6,9.8)
	Not Trained	8(9.8%)	36(73.5%)	.00	30.3	.00	27.3(8.1,91.6) *

1 = Indicate for Reference Group. * Significant association at P-Value < 0.05.

The multivariate regression analysis showed that there was no significant difference in the performance status of laboratory professionals in diagnosing malaria using smear microscopy with respect to age, sex, and experience of the study participants ($p > 0.05$) (Table 5).

4. DISCUSSION

Malaria is a major health challenge in Ethiopia, with recent increases in case numbers. Accurate diagnosis was crucial for effective malaria control. The final status of malaria diagnosis performance showed that 62.6% (CI 95%, 54.2–71%) of the results were acceptable, which was very low, while 37.4% (CI 95%, 29%-45.8%) were unacceptable. These results were lower than those reported in studies conducted in West Amhara, 91.8% (11); Uganda, 91% (18); and Ethiopia, 76% (23). However, our study was comparable to findings from eastern and central Oromia, 67.39% (28), and Western Oromia, 62.3%. The overall low agreement in malaria diagnosis performance might have been due to poor species agreement performance, very low agreement parasite count performance within the acceptable range,

the fact that the majority of participants were not performing parasite counts, lack of malaria smear microscopy training, and the educational level of participants.

The findings of this study demonstrated a parasite detection accuracy among laboratory professionals in West Oromia EQA centers, with an overall high-performance agreement of 95% (CI 95%, 91.3%-98.7%) (k = 0.91), sensitivity of 95.7%, and specificity of 96.2%. These results were comparable to previous studies conducted in Ethiopia, such as those from the Western Amhara region (97.31% agreement) (11). and Addis Ababa (91.7% agreement) (27). Furthermore, the current findings were higher than those from Hawassa (88% agreement) (20), Tanzania (87% agreement) (14), the Eastern and Central Oromia region (80.45% agreement) (28), Tigray (79% agreement) with sensitivity and specificity at 63% each (25), and West Oromia (78% agreement) (22). The observed increase in diagnostic accuracy among our study participants might have been related to the availability and proper use of standard operating procedures (SOPs) and external quality assurance (EQA) guidelines (both at 96.9%), 100% EQA participation, and regular quality control (QC) practices (73.2%), which significantly contribute to ensuring accuracy and consistency in results.

Despite the high detection accuracy, false negatives (3.8%) and false positives (4.3%) were observed, which could have led to misdiagnosis and inappropriate treatment. These were comparable to studies in the Eastern and Central Oromia region: false negative 4.04% and false positive 7.16% (28). The current findings were lower than those reported in Addis Ababa, which showed a false negative rate of 16.8% and a false positive rate of 24.4% (27), and in Western Oromia, with16.8% false negative and 9.9% false positive (22), where false reporting was linked to inadequate training and workload pressures.

Although parasite detection was high, species identification showed low agreement at 69% (CI 95%, 61.1%-77%) (κ = 0.52). The correct identification rate was highest for *P. vivax* (77.1%), followed by *P. falciparum* (74.4%), while mixed infections were poorly identified (42.7%). Misclassifications were common, with 18.3% of *P. falciparum* slides reported as mixed infections and 36.6% of mixed infections misidentified as *P. falciparum*. This species identification agreement was comparable to findings from studies conducted in Hawassa (74.3%) (20), Addis Ababa (67.63%) (27), and the Eastern and Central Oromia region (63.03%) (28). In contrast, it was higher than reports from West Oromia (63.03%) (22) and Addis Ababa and surroundings (51.1%) (26). Also, the finding was lower than studies conducted in the Western Amhara region (94.6%) (11), Tigray (77%) (25), and West Oromia (44.6%) (28). The difficulty in correctly identifying mixed infections—often confused with mono-infections—was a recurring issue, as shown by 36.6% of mixed infections being misdiagnosed as *P. falciparum* and 20.7% as *P. vivax* in this study. Our findings align with studies in Ethiopia (24; 28) and Tanzania (14), where species differentiation was a persistent challenge. The low accuracy in mixed infection detection is concerning, as misdiagnosis could have led to inappropriate treatment regimens, particularly in areas where *P. falciparum* and *P. vivax* coexist. The higher misclassification of mixed infections as *P. falciparum* (36.6%) suggested a tendency to overdiagnose the more severe species, possibly due to limited exposure to mixed infections or inadequate training.

This study revealed a significant challenge for laboratory personnel in parasite quantification. Only 3.4% (CI 95%, 0.3%-6.5%) of participants accurately estimated parasite density, which was lower than findings from Tanzania (17.8% correct quantification) (14) and Addis Ababa and surroundings (7.5% correct quantification) (26). This parasite quantification performance was comparable to findings from studies conducted in Western Oromia, where 3.3% of participants correctly quantified the slide (22). In this study, the very low performance of quantification of malaria parasites by the laboratory professionals might have been related to most study participants not performing parasite counting in their routine diagnosing of malaria smear microscopy and those practicing parasite quantification.

Participants used the less precise, plus-semi quantitative (+, ++, +++) system rather than standardized quantification methods per microliter or per WBC count. This aligned with findings from other studies, where very few participants accurately quantified parasite loads, and many did not report parasite density at all, as observed in Tigray (25) and Eastern Oromia (28), where poor parasite density reporting was linked to inadequate training and the absence of standardized protocols. This low adherence to WHO-recommended quantification standards highlighted a critical gap in diagnostic quality that could have affected treatment decisions and disease monitoring.

In this study, training status was the strongest predictor of competency (AOR = 27.3, $p < 0.001$), with untrained professionals performing significantly worse. BSc & above holders were 4.87 times more likely to be competent than diploma holders (AOR = 4.87, $p < 0.05$). This finding was higher than studies in the Eastern and Central Oromia region (5.12 times) (28), Tigray (7 times) (25), Kenya (13.8 times), and West Oromia (16.95 times) (22), which demonstrated the positive impact of training on diagnostic performance, making them more likely to provide high-quality diagnoses. These training interventions significantly improved performance.

Interestingly, the study also found that nearly all laboratories had available SOPs, participated in EQA programs, used both thick and thin films, performed quality control, used functional microscopes, and scanned at least 100 fields before reporting negative results. All of these are crucial quality indicators for malaria microscopy and may have contributed to the accurate detection rates observed.

Regarding WHO proficiency testing, the majority of participants (54.2%) demonstrated "very good" performance, primarily due to errors in species identification and parasite density estimation. This is consistent with studies in the U.S. (3), where unacceptable responses were higher for *P. ovale* (20.6%) and *P. malariae* (10.9%), indicating that even in well-resourced settings, species differentiation remains challenging. These findings reinforce that despite strong detection ability, there is a performance gap in the areas of identification and quantification, in line with national and regional studies (24; 27; 25).

5. Conclusion

The final acceptable proficiency test result of laboratory professionals was 62.6% and demonstrated high accuracy in parasite detection (95% agreement, 95.7% sensitivity, 96.2% specificity); however, species identification and parasite quantification remained major weaknesses. The moderate agreement

in species identification (69%) and the extremely low accuracy in parasite counting (3.4%) indicated significant gaps in the competency of laboratory professionals. The high diagnostic reliability for detecting parasite presence suggested effective adherence to standard operating procedures (SOPs) and participation in external quality assurance (EQA) programs. Training status and educational level were key predictors of the performance of laboratory professionals in diagnosing malaria smear microscopy.

Abbreviations

DHIS2: District Health Information System 2; EPHI: Ethiopia Public Health Institute; EQA: External Quality Assessment; ETB: Ethiopian Birr; FMOH: Federal Ministry of Health; GTS: Global Technical Strategy; PHEM: Public Health Emergency Management; PI: Principal Investigator; Pf: *Plasmodium Falciparum*; Pv: *Plasmodium Vivax*; PT: Proficiency test; QA: Quality Assurance; QBC: Quantitative Buffy Coat; RDT: Rapid Diagnostic Test; SOP: Standard Operating Procedure; SPSS: Statistical Package for the Social Sciences; WHO: World Health Organization

Declarations

Ethics approval and consent to participate

A letter of ethical clearance was obtained from the Research Ethics Committee of Wallaga University, Institute of Health Sciences. The official letter was written with the reference number (IHSREC006/052/2025). Consent was obtained from all the study participants after providing brief information about the objectives and the aim of the research. In addition, all study participants were informed that participation in this study relies on their will; no one can force them to participate in the study. Finally, the confidentiality of the information gathered was assured to the self-administered questioner by avoiding the name and address in the questionnaire.

Consent for publication

Not Applicable.

Availability of data and materials

All relevant data are within this paper and data for this study will be available upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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

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Figures

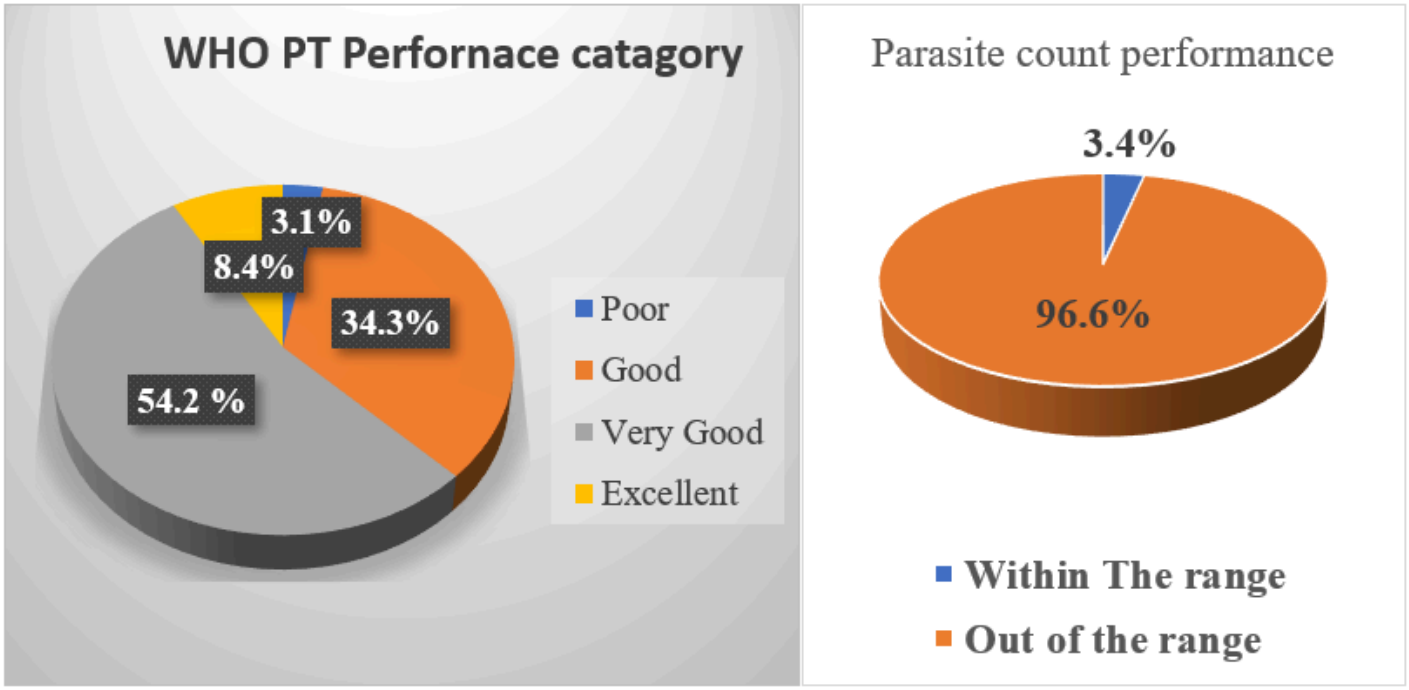


Figure 1

WHO Malaria PT performance category and parasite count.

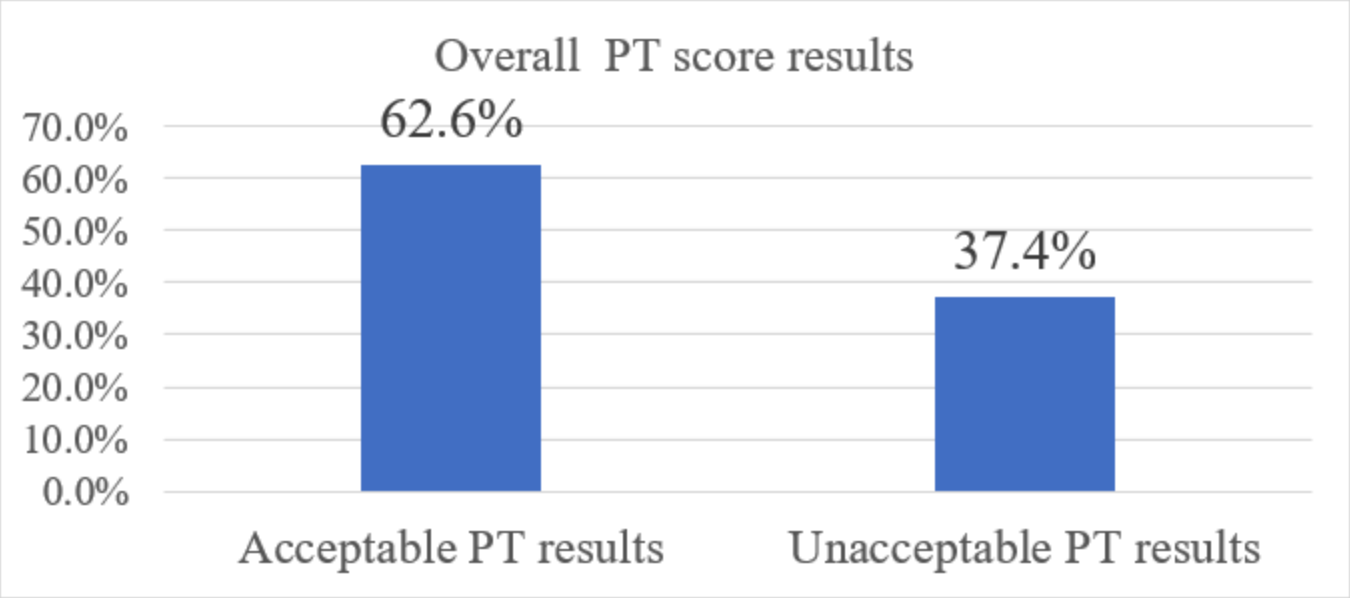


Figure 2

Overall percent of final PT score among the participants, west Oromia Malaria EQA center 2025.