

WALLAGA UNIVERSITY, INSTITUTE OF HEALTH SCIENCES



**DEPARTMENT OF MEDICAL LABORATORY SCIENCE
POSTGRADUATE PROGRAM**

**TITLE: PERFORMANCE ASSESSMENT OF LABORATORY
PROFESSIONALS ON MALARIA SMEAR MICROSCOPY AND
ASSOCIATED FACTORS IN WEST OROMIA MALARIA EXTERNAL
QUALITY ASSESSMENT CENTERS, ETHIOPIA 2024/2025**

GETU SILESHI ABDISA (BSc)

**A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL
PARASITOLOGY, WALLAGA UNIVERSITY IN, PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN MEDICAL PARASITOLOGY**

June, 2025

NEKEMTE, ETHIOPIA

WALLAGA UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY

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ON MALARIA SMEAR MICROSCOPY AND ASSOCIATED FACTORS IN
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CENTERS, ETHIOPIA 2024/2025**

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NEKEMTE, ETHIOPIA

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By my signature below, I Mr Getu Sileshi Abdisa hereby declare and affirm that this thesis entitled **“Performance assessment of laboratory professionals on malaria smear microscopy and associated factors in West Oromia Malaria External Quality Assessment Centers, Ethiopia 2024/25”** is my conducted under the supervision of Dr. Geletta Tadele (PhD) and Mr. Kinfu Boresa (MSc). I have followed all ethical principles of scholarship in the preparation, data collection, data analysis, and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I affirm that I have cited and referenced all sources used in this document. Every effort has been made to avoid plagiarism in the preparation of this thesis.

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Abstract

Background: External Quality Assessment (EQA) allows health facilities to assess their capabilities by comparing their results with those of other laboratories in the network. Maintaining a proper setting and competency standards for laboratory personnel to ensure skills and best practices are vital to malaria microscopy performance.

Objective: This study evaluated the performance assessment of laboratory professionals in diagnosing malaria smear microscopy and associated factors in West Oromia 2024/25 Malaria External Quality Assessment Centers.

Methods: A cross-sectional study design was employed among 131 laboratory professionals working at 25 Malaria EQA centers in the west Oromia region from January to March 2025. Information on demographic characteristics was collected using a self-administered questionnaire. A total of 10 pre-validated panels of malaria slides were used to assess laboratory professionals' performances on detection, differentiation of common species of malaria, and quantification of parasite density. Data were entered and cleaned using Epidata, then analyzed using SPSS version 20. Descriptive statistics were used to summarize the findings, and diagnostic performance metrics were calculated. Variables with a p -value < 0.25 in the bivariate analysis were included in the multivariate logistic regression, with a p -value < 0.05 considered statistically significant.

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 competency of laboratory professionals in diagnosing malaria smear microscopy.

Conclusion and Recommendation: The study found that 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 level and recent malaria training. Training and promoting higher education among laboratory staff were important to enhance diagnostic accuracy of malaria microscopy in West Oromia.

Keywords: Performance Assessment, Smear Microscopy, Malaria Microscopy, External Quality Assessment (EQA).

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Acronyms and 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

CHAPTER ONE: INTRODUCTION

1.1 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).

Ethiopia has a total area of 1.1 million square kilometers. Ethiopia's topographical features range from peaks as high as 4,550 meters (m) above sea level in Ras Dashen to 110 m below sea level in the Afar Depression (5). Additionally, Ethiopia's highlands are separated into two sections by the Great East African Rift Valley: the northwest and the southeast highlands. Due to the above-mentioned geographical features as well as the ecological and climatic differences, the country has a diverse eco-epidemiology that favors heterogeneous malaria transmission (6).

The malaria life cycle involves two hosts: humans and mosquitoes. Human Host (Asexual Cycle): Liver stage (exo-erythrocytic schizogony): Sporozoites from mosquito saliva invade hepatocytes, multiply, and release merozoites. Blood stage (erythrocytic schizogony): Merozoites infect red blood cells (RBCs) and undergo cycles of multiplication (trophozoites → schizonts → rupture), causing clinical symptoms. Gametocyte formation: Some parasites develop into sexual forms

(gametocytes), which are taken up by mosquitoes during a blood meal. Mosquito Host (Sexual Cycle): Gametocytes mature into gametes in the mosquito gut, fuse to form zygotes, and develop into sporozoites that migrate to salivary glands, ready to infect another human (7).

The pathogenesis of malaria is primarily linked to the destruction of red blood cells and the host's immune response to the parasite. Infected erythrocytes adhere to vascular endothelium, leading to blockages in microcirculation, which can cause severe complications such as cerebral malaria and multi-organ failure. Pathophysiology of malaria. Clinically, malaria presents with nonspecific symptoms such as fever, chills, headache, malaise, nausea, and vomiting. Severe cases may progress to cerebral malaria, acute renal failure, hypoglycemia, severe anemia, and death if not promptly treated. Children under five, pregnant women, and immunocompromised individuals are at higher risk of complications (8).

In Ethiopia, around 68% of the total population lives in malaria-prone areas. It is the most common infectious disease in the country and is responsible for about 30% of all disability-adjusted life years lost. In recent years, it has repeatedly been cited as one of the three main causes of morbidity and mortality (9). The pooled prevalence of malaria among adults in Ethiopia was 13.61%. Subgroup analysis based on types of malaria cases showed malaria prevalence among symptomatic and asymptomatic adults was 15.34% and 11.99%, respectively, and the highest malaria prevalence was recorded in Oromia Regional State (13.11%) (10). A clear diagnosis based on clinical symptoms is impossible, as many signs and symptoms overlap with those of other febrile illnesses. Therefore, 100% of suspected cases must be diagnosed in a laboratory within 24 hours of the onset, according to Ethiopia's national strategic plan for malaria (11).

In a laboratory setting, the identification of malaria is conducted through various methodologies, including traditional microscopic diagnosis achieved through the staining of both thin and thick peripheral blood smears, along with additional concentration methodologies like molecular diagnostic approaches, rapid diagnostic tests (RDTs), and the quantitative buffy coat (QBC) technique (12).

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. The procedure

includes several steps: Blood Film Preparation: Both thick and thin smears are prepared. The thick film concentrates the parasites and is used for parasite detection due to higher sensitivity; RBCs are lysed during staining, concentrating parasites, while the thin film preserves parasite morphology. Used for species identification, RBCs are spread in a single layer, fixed with methanol, and stained. Fixation: Thin films are fixed with absolute methanol to preserve cell morphology. Thick films are air-dried without fixation to allow hemolysis of red blood cells during staining. Staining: Giemsa stain is the most commonly used dye for malaria microscopy. It stains the parasite's cytoplasm blue and chromatin red, providing contrast for easier detection. Proper staining requires buffered water (pH 7.2) and precise timing (typically 10 minutes for thin films and 15–20 minutes for thick films). Systematic Examination: Thick film Microscopy is performed under oil immersion using a 100× objective for parasite detection. Thin film: Examined at 100x for species identification based on RBC morphology, parasite stages, and staining characteristics. Parasite density is calculated per microliter of blood, aiding in treatment monitoring. A systematic approach includes scanning the entire smear in a zigzag pattern to avoid missing parasites. For negative results, at least 100 high-power fields should be examined before declaring the smear parasite-free (12).

The World Health Organization advises the verification of blood slides through cross-checking. A selection of routine blood slides is forwarded to a reference laboratory for accuracy assessment. External Quality Assessment (EQA) programs present another method for ensuring quality. In these programs, the reference laboratory dispatches stained blood film samples to peripheral laboratories, which analyze the samples and provide a report. Subsequently, they receive feedback regarding the correct results and their performance (13). The primary factors contributing to the ineffectiveness of the existing malaria control strategy are the shortage of qualified professionals capable of diagnosing malaria and the absence of consistent quality control measures within the laboratory diagnostic process (14). Although it requires highly skilled personnel, a functioning laboratory, and a regular supply of reagents, routine parasitological diagnosis of malaria in blood films by light microscopy remains the gold standard that can be used for species differentiation, quantification of parasites, management of severe disease, and investigation of treatment failures. Maintaining an appropriate environment and standards of competence of laboratory staff is essential for performing malaria microscopy (6).

Participating labs can assess their capabilities through EQA by contrasting their outcomes with those of other labs in the network. This can be done through panel testing (PT) or blinded follow-up of slides for microscopy, as well as a review of laboratory performance through on-site monitoring for both microscopy and RDTs. Maintaining an appropriate framework and competency standards for laboratory staff to ensure that skills and best practices are an integral part of microscopic malaria performance (15).

1.2 Statement of Problem

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.

1.3 Significance of the Study

The significance of this study lies in addressing the existing gaps in the quality of malaria microscopy diagnosis, which is crucial for accurate detection, species identification, and parasite load quantification. Despite the documented evidence of performance gaps, there is limited research in the study setting on the factors affecting malaria diagnosis accuracy. By assessing these factors, the study aims to enhance the effectiveness of laboratory professionals and reduce diagnostic errors, ultimately leading to more reliable malaria detection.

The findings will be valuable to policymakers, the Ministry of Health, and regional health bureaus in designing strategies to improve laboratory services. The study's results will inform targeted interventions, such as professional development programs, ensuring the availability of adequate resources and strengthening quality assurance systems. These improvements will lead to better capacity-building efforts, helping laboratory professionals deliver accurate and consistent results, which are critical for malaria control and treatment.

Ultimately, this research will contribute to the continuous improvement of malaria microscopy diagnosis in the region. By identifying and addressing the factors that influence diagnostic performance, the study will support ongoing efforts to enhance malaria management and control, improving public health outcomes. The insights gained will be essential in the development of sustainable solutions for the detection and treatment of malaria, benefiting both health professionals and patients in the study area.

CHAPTER TWO: LITERATURE REVIEW

2.1 Performance on Laboratory Detection of Malaria Parasites

A research study conducted in Tanzania evaluated the precision of microscopic malaria diagnosis. The findings indicated that laboratory professionals at health facilities demonstrated an 87% concordance with reference readers in identifying malaria parasites. The overall sensitivity and specificity of the health facility professionals were recorded at 84.3% and 90.8%, respectively. This study revealed significant discrepancies in the accuracy of malaria diagnoses among different facility readers (14).

A study conducted in Uganda assessed the accuracy of malaria microscopy in private health facilities in Entebbe Municipality, resulting in an overall agreement of 91.0%; sensitivity was 95.8%, and specificity was 90.1% (18).

A study on mentorship for malaria microscopy diagnostic services in Ethiopia assessed the baseline competencies of microscopists and the performance of health facilities. In terms of proficiency testing, the malaria microscopists demonstrated an overall percent agreement of 91.8% in detecting parasites. The sensitivity for malaria parasite detection was recorded at 92.7%, while the specificity was noted to be 89.1% (23).

A study on the comprehensive evaluation of competency among malaria microscopists and the capacity of laboratory diagnostic services in Ethiopia found that the agreement rate for parasite detection was 84.6%, with a sensitivity of 83.2% and a specificity of 90.1% (24). A similar study on the performance evaluation of laboratory professionals on malaria microscopy conducted in Hawassa Town, Southern Ethiopia, reported the agreement rate (88%), sensitivity (82%), and specificity (96.5%) (20).

In Tigray, Northern Ethiopia, a study on the performance of laboratory professionals in malaria microscopy found an overall agreement of 79% in parasite detection, with a sensitivity of 63% and specificity of 95.7% (25). A study in the West Amhara region on the performance of malaria

microscopy external quality assessment (EQA) and networking among health facilities reported a test agreement of 97.31% in parasite detection (11).

A study conducted in Addis Ababa and its surrounding areas on the performance assessment of malaria microscopists at defense health facilities found a 71.4% agreement in parasite detection between study subjects and expert readers, indicating "fair agreement." The overall sensitivity was 65.7%, and specificity was 100% (26). Another study in Addis Ababa on the assessment of malaria microscopic diagnosis performance among laboratory professionals in public health facilities reported a 91.7% agreement in parasite detection (27).

A study in the Eastern and Central Oromia Region evaluating malaria microscopy diagnosis performance in public hospitals found an overall agreement of 80.45%, with a sensitivity of 77.63% and a specificity of 96.41% (28). Similarly, a study in West Oromia on external quality assessment of malaria microscopy diagnosis in selected health facilities reported an overall agreement of 78% in parasite detection, with a sensitivity of 77% and a specificity of 83.3% (22).

2.2 Performance on Plasmodium Species Identification

A study on the comprehensive competency assessment of malaria microscopists and laboratory diagnostic service capacity in Ethiopia found that participants had a slight agreement (43.8%) in differentiating *Plasmodium* species in single/mixed infections. Only 28.41% correctly differentiated *P. falciparum* from other species (24). Another study on mentorship for malaria microscopy diagnostic services in Ethiopia reported a slight agreement (57%) in species identification (23).

In Hawassa Town, Southern Ethiopia, a study evaluating laboratory professionals' performance in malaria microscopy found a lower agreement (74.3%) in identifying mixed infections (20). In contrast, a study in Tigray, Northern Ethiopia, reported an 87% overall agreement in species identification (23). A study in the Western Amhara region on external quality assessment (EQA)

of malaria microscopy and health facility networking found a species identification agreement of 94.6% (11).

A study in Addis Ababa assessing malaria microscopists' performance at defense health facilities reported a 51.1% agreement in species identification, indicating slight agreement (26). Another study in Addis Ababa on malaria microscopic diagnosis performance in public health facilities found a 67.63% agreement in species identification (27).

In the Eastern and Central Oromia Region, a study evaluating malaria microscopy diagnosis performance in public hospitals found a 63.03% agreement in species identification. Among misdiagnosed cases, 28.2% of mixed infections were reported as *P. falciparum*, and 23.5% of *P. vivax* cases were misidentified as *P. falciparum* (28). A study conducted in West Oromia public health facilities by laboratory professionals on malaria microscopy identification of plasmodium species with reference readers was 44.6% (22).

2.3 Performance on Laboratory Quantification of Parasite Load.

A study in Tanzania found that only 17.8% of parasite-positive slides were accurately assessed for parasite density (within $\pm 25\%$ of expert-measured density). Over 50% of low-to-medium-density slides were misclassified as high density, while fewer than 50% of high-density slides were correctly identified. At the hospital level, all high-density slides were correctly identified, but low-to-medium-density slides were misclassified as either high density or negative (14).

In Tigray, Northern Ethiopia, 91.3% of laboratories used a semi-quantitative "plus system" to estimate parasite load. While 63% correctly identified the species on Slide A, only 10.9% reported the correct density (++++). Slide B had a correct density report from only 15.2% of participants, and Slide C was correctly identified by just 6.5%, with only 2.2% accurately reporting its density. Only 15.2% of participants correctly diagnosed the mixed infection in Slide E (29).

In the Eastern and Central Oromia Region, 52.2% of laboratory professionals did not report parasite density for positive cases, and among those who did, 33.3% made incorrect quantifications (28). In West Oromia, only 3.3% of professionals correctly quantified parasites in all positive slides, while 40% did not report density, and 56.7% correctly quantified at least one slide (22).

2.4 Factors with Laboratory Professional's Malaria Diagnosis Performance

A study in Nigeria assessed medical laboratory scientists' performance after a 10-day malaria microscopy training. The mean post-test score for parasite counting improved from 4.2% to 27.9% (30).

In Ghana, medical laboratory professionals' proficiency in parasite detection improved from 64% to 87% after training. Species identification accuracy increased from 17% to 78%, and overall microscopy skills nearly doubled post-training (31).

A study in Kenya showed that professionals who received refresher training were 13.8 times more likely to make accurate malaria diagnoses than those who had not. Similarly, those with more than five years of experience were 3.8 times more likely to provide accurate diagnoses than their less-experienced counterparts (32).

In Tigray, laboratory personnel who took refresher training were 7 times better in parasite detection and identification than untrained staff (25). A study in Addis Ababa found higher misdiagnosis rates among untrained professionals, though work experience and qualifications showed no significant association (27).

A study in the U.S. on proficiency testing (PT) for malaria diagnosis found that laboratories performed best in detecting *P. falciparum* (3.4% unacceptable responses), followed by *P. vivax* (9.0%), *P. malariae* (10.9%), and *P. ovale* (20.6%) (33).

A study in Northwest Ethiopia examined whether routine microscopy for malaria detection and quantification met national standards. It found that 96.6% of participants had prior experience in species identification and quantification, but nearly half used both thick and thin films inconsistently (34). In the Eastern and Central Oromia Region, professionals with >5 years of experience were 10.56 times more proficient in malaria detection/identification than those with ≤5 years. Trained personnel produced results 6.12 times more accurate than untrained staff (28). In West Oromia, trained professionals were 16 times more likely to provide accurate malaria microscopy diagnoses (22).

2.5 Conceptual Framework(1).

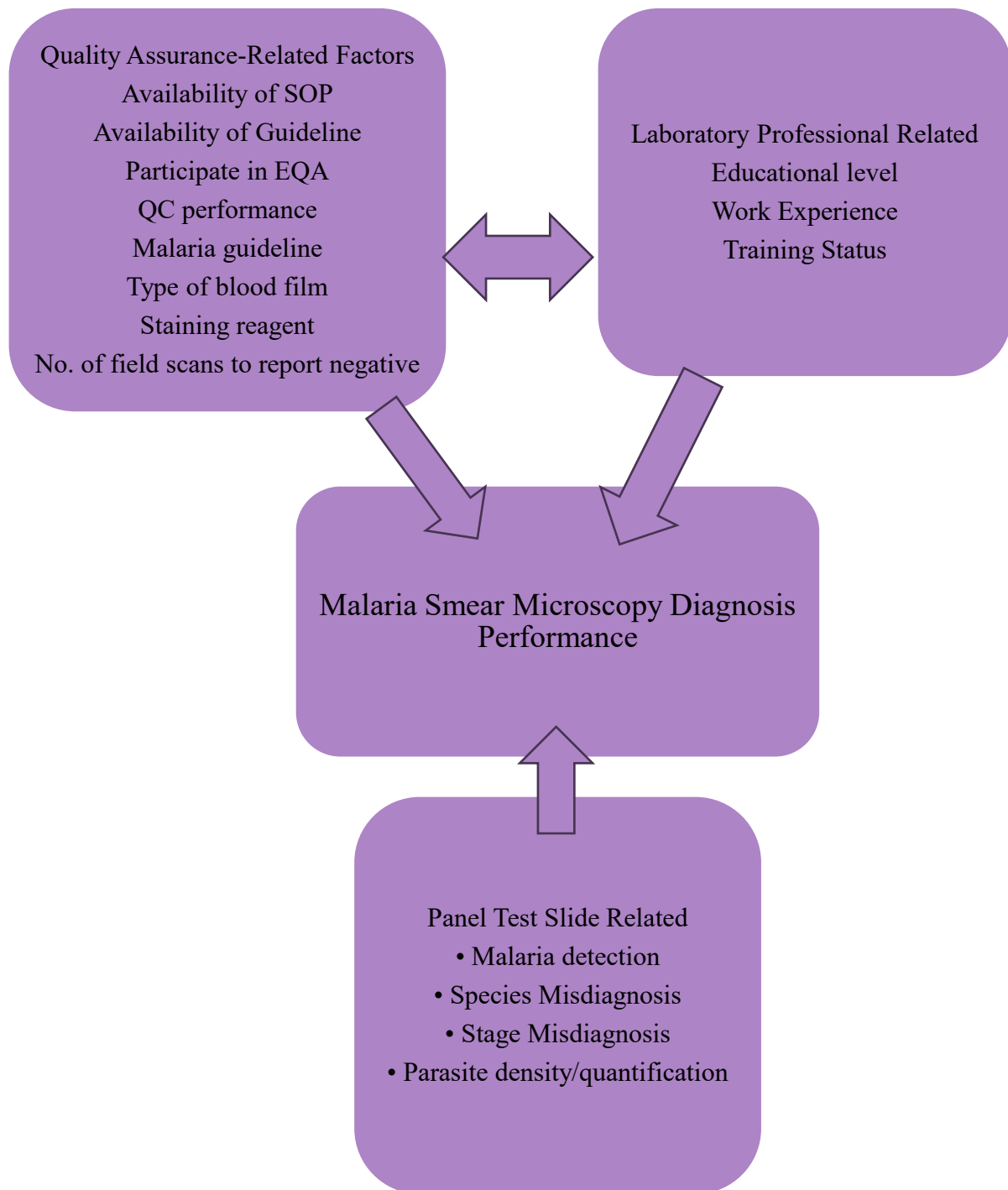


Figure 1: Conceptual Framework

CHAPTER THREE: OBJECTIVES

3.1 General Objective

- ❖ To assess the performance status of laboratory professionals in diagnosing malaria smear microscopy and associated factors in West Oromia Malaria EQA Center hospitals.

3.2 Specific Objectives

- ❖ To determine the level of performance of the laboratory professionals on parasite detection, species identification, and parasite load counts in West Oromia malaria EQA Centers.
- ❖ To determine the factors associated with the performance of laboratory professionals in West Oromia Malaria EQA centers in the diagnosis of malaria smear microscopy

CHAPTER 4: METHODS AND MATERIALS

4.1 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.

4.2 Study Design

A hospital-based cross-sectional study design was undertaken to assess the performance assessment of laboratory professionals in diagnosing malaria smear microscopy and associated factors by distributing an equal number of uniformly prepared Giemsa-stained malaria slides produced by the National Malaria Slide Bank, and the slides were validated by Level 1 & 2 WHO-certified professionals at the Nekemte Public Health Research and Referral Laboratory Center.

4.3 Population

4.3.1 Source Population

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

4.3.2 Study Population

A simple random sampling to select study participants. Data were collected by distributing a standardized, pre-validated malaria slide panel and self-administered questionnaires to assess the ability of laboratory professionals to detect malaria parasites in terms of sensitivity and specificity,

differentiation of common species of malaria, correct reporting of parasite density and proportion of major and minor errors in diagnosis of malaria among study participants, and associated factors.

4.4 Eligibility Criteria

4.4.1 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.

4.4.2 Exclusion Criteria

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

4.5 Sample Size Determination and Procedure

4.5.1 Sample Size Determination

The sample size was determined using the standard formula: $n = [z^2 p(1-p)] / e^2 / 1 + [z^2 p(1-p)] / e^2 * N$. and a 95% confidence level, 0.22 (35). The malaria microscopy percentage of agreement was 78%, as previously reported in West Oromia (22). and a marginal error of 5%. Thus, a sample size of 131 will be calculated. Population size = 260

Sample size formula $n = [z^2 p(1-p)] / e^2 / 1 + [z^2 p(1-p)] / e^2 * N$

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.

Table 1: Sample size determination for objective on factors associated with the performance of laboratory professionals in the diagnosis of Malaria smear microscopy

Relevant Factors	Power	Assumption						Reference
		$Z \frac{\alpha}{2}$	% among Exposed	% among Unexposed	Ratio	AOR	SS	
Training Status	80%	1.96	65.2	34.8	0.53	6.12	96	(2)
Work Experience	80%	1.96	45.7	54.3	1.19	10.56	50	(2)

The large sample size used

4.5.2 Sampling Procedure

By using a simple random method to select the study population, microscopists from the selected hospitals obtained the study population by using the lottery method by writing 1 and 0 for participants who could pick number 1 to participate in the study in this health facility.

4.6 Data Collection Procedure

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. 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 with closed-ended questions were utilized by laboratory professionals. When the microscopists had completed reading the slide panels in the

given time, they wrote on the result reporting form for reading the malaria slide panel test, and the slides were collected back to their slide box for administration in other facilities when the data collection teams were moving.

4.7 Data Collection Tool

4.7.1 Malaria slide panels

Slide panels were used to test the performance of malaria microscopists on parasite detection, species identification, and quantification. An equal number of uniformly prepared Giemsa-stained malaria slides were produced by the National Malaria Slide Bank by WHO Level 1 certified professionals, and when preparing each slide, parasite load was categorized into low parasitemia, medium parasitemia, and high parasitemia, and for each category, there were a lower limit, a mean, and an upper limit. Standardized malaria slide panels were distributed to the species, and mean parasitemia was as follows:

Table 2: The panels included 10 slides for diagnosis.

Slide No.	Result	Parasitemia	Lower Limit	Upper Limit	Mean
Slide 1	<i>P. falciparum</i>	Low Parasitemia	10,609/ μ l	17,682/ μ l	14,146/ μ l
Slide 2	<i>P. falciparum</i>	High parasitemia	49,554/ μ l	82,591/ μ l	66,073/ μ l
Slide 3	<i>P. Vivax</i>	Low Parasitemia	10,609/ μ l	17,682/ μ l	14,146/ μ l
Slide 4	<i>P. Vivax</i>	Medium parasitemia	18,018/ μ l	30,028/ μ l	24,023/ μ l
Slide 5	mixed (<i>P. falciparum</i> and <i>P. Vivax</i>)	Medium Parasitemia	15,534/ μ l	25,888/ μ l	20,711/ μ l
Slide 6,7,8,9 &10	Negative				

4.7.2 Study questionnaires

Structured questionnaires were used to collect information on the participating facilities and socio-demography of professionals, including age, sex, educational status, diagnostic experience, and refreshment training.

4.8 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.

4.9 Study Variables

4.9.1 *Dependent Variable*

- Malaria smear microscopy diagnosis status.

4.9.2 *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

4.10 Operational Definition

Acceptable: Achieves a PT score of 80% or more.

Unacceptable: Fails to reach the PT score of < 80%.

Excellent: $\geq 90\%$ correct identification of malaria species, stages, and parasite density.

Very Good: $80\text{--}< 90\%$ correct identification of malaria species, stages, and parasite density.

Good: $70\text{--}< 80\%$ correct identification of malaria species, stages, and parasite density.

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

4.11 Data Analysis Technique

Table 3: Interpretation of Malaria PT Results(36).

Key Diagnostic Criteria	Value
Positive slide reported as negative	0 points per slide
Negative slide reported as positive	0 points per slide
Positive slide reported correctly as positive	3 points per slide
Positive slide reported with correct parasite species identification	3 points per slide
Positive slide reported with correct parasite stage identification	2 points per slide
Positive slide reported with correct parasite load	2 points per slide
Negative slide report correctly as negative	10 points per slide

Table 4: 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 $\pm 25\%$ of the mean is acceptable (36).

After the completeness and consistency of data were checked by the principal investigator, data were entered & cleaned by Epidata and analyzed using SPSS for Windows version 20. Descriptive statistics was 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. Based on WHO recommendations concerning the percentage agreement in readings between the participants and expert microscopists, malaria microscopists were classified into four categories: “Poor” ($<70\%$ agreement), “Good” ($70\%—<80\%$), “Very good” ($80\%—<90\%$), and “Excellent” ($>90\%$). Those categories were summarized into two: Excellent and Very Good ($>80\%$), Acceptable for the category Good, and Poor ($<80\%$), unacceptable (20). The Kappa value was calculated to see the strength of the agreement.

4.12 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. 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.

4.13 Dissemination Plan

The result of the study will be disseminated to the Wallaga University Department of Medical Laboratory, the Oromia Health Bureau, the Nekemte Public Health Research and Referral Laboratory Center, the Ministry of Health, presentations at conference, partners supporting the malaria elimination strategy, and publications in health journals.

CHAPTER 5: RESULTS

5.1 Sociodemographic Characteristics of Study Participants

The majority of the study participants were less than 30 years old. A higher proportion of participants was male. Most participants hold a BSc & above and have more than 2 years of work experience; 47.3% of the participants were trained within the past 3 years. All microscopes were functional; the most commonly used microscope was the Olympus brand. 94.7% of participants used both thick and thin films for malaria diagnosis. 71.8% of laboratory personnel did not perform routine parasite counts. 67.6% of laboratory personnel who did parasite counts utilized the semi-quantitative quantification (+, ++, +++) method (Table 5).

Table5: Socio-demographic characteristics of laboratory professionals who participated in the performance evaluation of malaria smear microscopy in West Oromia Malaria EQA Center.

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
	Percent of RBC infected	0	0

5.2 Quality Assurance and Diagnostic Practices in Malaria Microscopy

The majority, 96.9%, of laboratories have available SOPs, 96.9% reported having EQA (External Quality Assessment) guidelines, and all laboratory professionals participate in EQA. The majority participate in all types of EQA methods (onsite evaluation, RBR, and PT); 96.9% perform quality control of those performing QC, and 73.2% do so regularly. 73.3% use buffered water to prepare the stain, 98.5% scan ≥ 100 fields before reporting a slide as negative, and all laboratories that participated in the study report positive cases with species identification and parasite stage (Table 6).

Table 6: 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

5.3 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 7).

Table 7: Diagnosis performance of laboratory professionals on malaria smear microscopy in 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				

5.3.1 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 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 8).

Table 8: Species identification performance of laboratory professionals on malaria smear microscopy in West Oromia Malaria EQA Center 2025.

Participant Results	Expected Slide Result			Total	Agreement	Kappa
	P. F	P. V	Mixed			
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%)		

5.3.2 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" (figure 2).

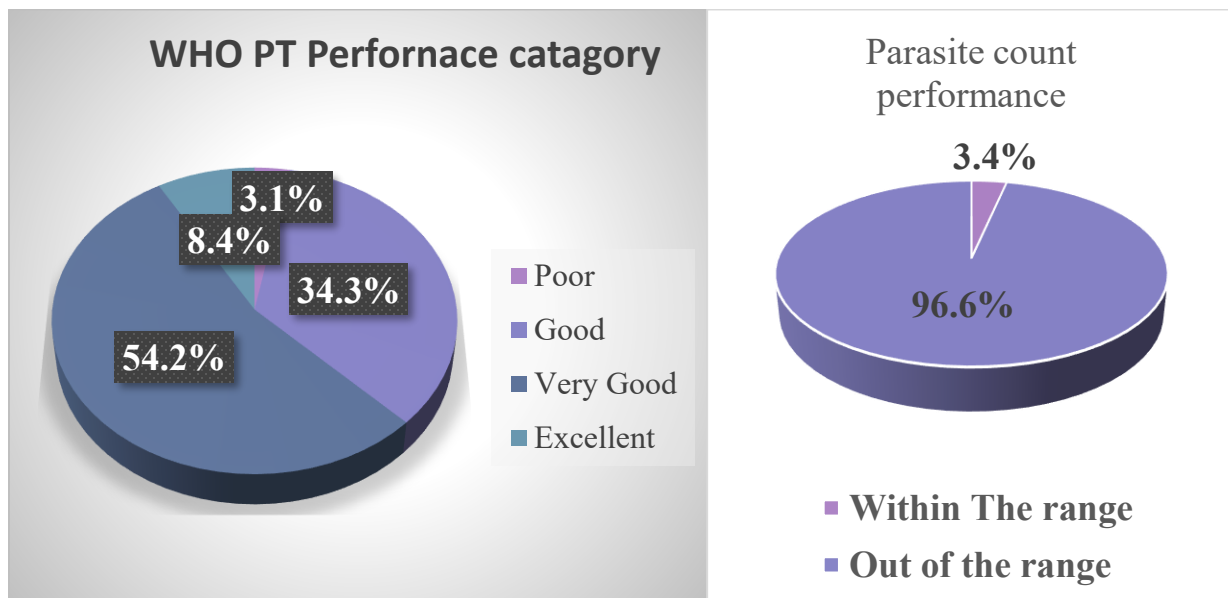


Figure 2: WHO Malaria PT performance category and parasite count.

The final overall PT result status was 62.6% acceptable (figure 3).

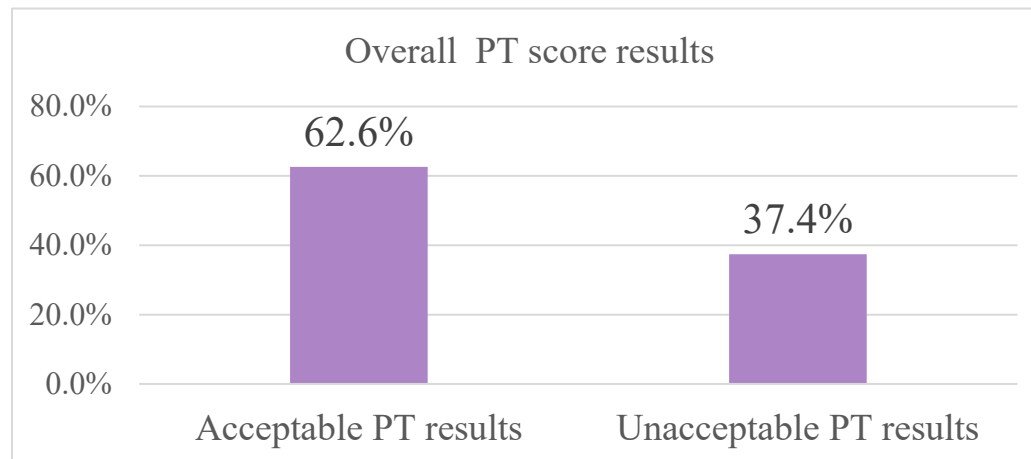


Figure 3: Overall percent of final PT score among the participants, west Oromia Malaria EQA center 2025.

5.4 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 9).

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 9).

Table 9: 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.

CHAPTER 6: 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 West 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, with 16.8% 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%) ($\kappa = 0.52$). 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 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).

Limitation

In the current study, we only used proficiency testing slides using unknown panels, which measure the best possible work or skill of a laboratory professional rather than routine or day-to-day performance in the diagnosis of malaria, since all participants may be well aware that they were examining external quality assessment samples. Furthermore, it was difficult to know professionals' performance in smearing and staining blood films for malaria diagnosis.

CHAPTER 7: CONCLUSION AND RECOMMENDATIONS

7.1 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.

7.2 Recommendation

The study confirms training and education level are critical for malaria microscopy competency.

- Regular refresher training should be prioritized, with a focus on species identification (particularly mixed infections) and WHO-recommended parasite quantification methods.
- Education-Based Recruitment and Support: Encourage higher education qualifications (BSc and above) for laboratory staff, and provide support for diploma holders to upgrade their qualifications.

By addressing these gaps, malaria diagnosis in West Oromia can be further improved, ensuring accurate detection, species identification, and parasite quantification—critical steps for effective malaria control and elimination efforts.

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CHAPTER 9: APPENDIX

Annex 1: Number of West Oromia EQA Center Functional Health Facility Profile, total lab staff, and 131 participants.

S/no	Zone	Health Facility Name	Total Lab Staff	Study Sample	Remark
1	Jimma	Limmu Genet General Hospital	10	5	
2		Shenen Gibe General Hosp	10	5	
3		Agaroo General Hospital	11	6	
4		Seka Primary Hospital	9	4	
5		Nada Primary Hospital	4	2	
6	I/A/Bora	Mattu Karl Comp Specialized Hospital	12	6	
7	B/Bedele	Bedele Genera Hospital	11	6	
8		Didessa Primary Hospital	2	1	
9	E/Wallaga	Nekemte Specialized Hospital	18	9	
10		Arjo Primary Hospital	10	5	
11		Sire Primary Hospital	10	5	
12	W/Wallaga	Gimbi General Hospital	14	7	
13		Ayira General Hospital	14	7	
14		Nedjo General Hospital	13	7	
15		Mendi Primary Hospital	9	4	
16	K/Wallaga	Dembi Dollo General Hospital	16	8	
17		Kake Primary hospital	11	6	
18		Hawa Gelan Primary Hospital	12	6	
19	H/G/Wal	Shambu General Hospital	8	4	
20		Guduru Primary Hospital	7	3	
21	W/Shoa	Ambo General Hospital	11	6	
22		Gedo General Hospital	11	6	
23		Guder Primary Hospital	8	4	
24		Bako Primary Hospital	10	5	
25		Enchini Primary Hospital	9	4	
			260	131	

Annex 2: Questionnaire

Performance assessment of laboratory professionals on malaria smear microscopy and associated factors in West Oromia EQA centers.			
No	Question	Response	Remark
1	Zone		
2	Woreda/Town		
3	Name Hospital		
4	Level of the hospital		
I. Factors Laboratory Professional Related Variables			
Code	Question	Response Category	Remark
101	Code (FC/PC*)	_____	
102	Age in Years	_____	
103	Sex	1. Male 2. Female	
104	The educational level of laboratory professionals	1. Diploma/Level 2. BSc degree 3. MSc	
105	Work experience	_____	
106	Have you training malaria diagnosis Training?	1. Trained < 3 years 2. Trained > 3 years 3. Not Trained	
107	Is the microscope functional	1. Yes 2. No	
108	If yes, which types of microscopes used	1. Olympus 2. Primo star 3. CX 4. Other Specify _____	
109	What method do you use for diagnosis of malaria?	1. Thin Film only 2. Thick Film Only 3. Thick and Thin Film	
110.	Do you perform Parasitemia Count?	1. Yes 2. No	
111	If yes, which methods do you use?	1. +, ++, +++ 2. Parasite per microliter/WBC 3. Parasite per micro/RBC 4. Percent of RBC infected	

II. Factors Quality Assurance Variable			
201	Availability of SOP	1. Yes 2. No	
202	Availability of EQA guideline	1. Yes 2. No	
203	EQA participation	1. Yes 2. No	
204	If yes, type of EQA	1. Onsite evaluation 2. RBR 3. PT 4. All	
205	Quality control performance	1. Yes 2. No	
206	If yes	1. Regularly 2. Not regularly	
207	Staining reagent	1. Write stain 2. Giemsa stain	
208	Use of buffered water to prepare Giemsa stain working reagent	1. Yes 2. No	
209	Minimum field scan to report negative	_____	
210	Report of positive results by species identification	1. Yes 2. No	
211	Report of the result by stage identification	1. Yes 2. No	

Annex 3: Result Reporting Form

Result reporting form for reading malaria slide panel test

Name of Health Facility _____

Code Lab technicians/technologists _____

Code	Slide ID	Result				PT Score 10%
		Negative	Positive			
			Species	Stage	Parasite Loadper Microliter/WBC	
301						
302						
303						
304						
305						
306						
307						
308						
309						
310						

Date: _____ Signature of the lab technician/technologist _____

Cumulative Score _____ % Grading Lab Performance _____ Acceptable Status _____

Annex 4A: Information sheet and consent form

1- Background to the study

Microscopic diagnosis is the best method for confirmation of malaria parasites; the careful preparation, fixation, and examination of thick and thin blood smears of blood samples on a slide is still the gold standard for the diagnosis of malaria. Currently, the performances of laboratories are poor due to inadequate equipment, insufficient training, and lack of supervision, skill of the technician, and quality assurance. The inability of laboratory professionals to identify malaria parasites or to confirm their absence on blood smears has led to the inappropriate administration of anti-malarial drugs for other febrile diseases. This is associated with an increased risk of developing drug resistance.

2. Why the study is important

Performance assessment of laboratories in the diagnosis of malaria helps to identify any gaps that lead to misdiagnosis of malaria and their associated factors and also helps to know how to improve the performance of the laboratories.

3. What are the risks of participating in the study?

There is no risk related to participating in the study.

4. If you don't want to participate or discontinue

The information collected will not have any specific information, including a name, that might break your anonymity. You have the right not to participate in the study or withdraw from the study at any time. You are also not forced to tell anything you don't want to answer regarding yourself. If you have any questions, you may consult me at the following addresses:

Getu Sileshi Abdisa (PI)

Department of Medical Laboratory Sciences, Institute of Health Science,

Wallaga University

Nekemte, Ethiopia.

Email: filenbarga2@gmail.com

Tel: +251917835430

Signature: _____

Date: _____

5- Consent form

I, the undersigned, have read the provided information. I understood that the study would be without major health risks to me and agreed to participate in the study.

Signature of participant: _____

Annex 4 B (Afan Oromo): Waraqaadeeffannoo fi unkahayyamaa

1- Seenaa qorannichaa

Qorannoo maaykirooskoopiiitiin paarasyitii busaa adda basuu mala hundarra gaarii ta'ee fi paarasayitii busaa mirkaneessuu dha; dhiiga islaayidii irratti of eeggannoodhaan qopheessuu, dhaabuu ykn maxxansuu fi qorachuun dhiiga furdaa fi haphii saamuda dhiigaa islaayidii irratti dibame ammallee sadarkaa ulaagaa warqee dhukkuba busaa adda baasuu dha. Yeroo ammaa kana meeshaaleen gahaa dhabuu, leenjii gahaa dhabuu, fi hanqina to'annoo, dandeettii teeknishaanotaa, fi mirkaneessa qulqullinaa irraa kan ka'e raawwiin laaboraatoorii gadhee ta'uu ibsameera. Ogeeyyiin laaboraatoorii paraasitii busaa adda baasuu ykn dhiiga irratti akka hin jirre mirkaneessuu dadhabuun isaanii dhukkuboota ho'a qaamaa biroof qorichi farra busaa sirnaan akka hin kennamne taasiseera. Kunis carraa qoricha ofirraa ittisuu dabaluu wajjin walqabatee jira.

2- Qo'annoon maaliif barbaachisaa ta'e

Raawwii Hojii Madaalliin laaboraatooriiwwan dhukkuba busaa adda baasuu keessatti qaawwa dhukku babusaa dogoggoraan adda baasuu fi wantoota isaan waliin walqabatan kamiyyuu adda baasuuf kan gargaaru yoo ta'u, akkasumas raawwii laaboraatoorii akkamitti akka fooyyessuu danda'amu beekuuf gargaara.

3- Qorannicha irratti hirmaachuun balaawwan maal fa'a?

Qorannicha irratti hirmaachuu waliin balaan wal qabatu hin jiru.

4- Yoo hirmaachuu ykn addaan kutuu hin barbaanne

Odeeffannoon walitti qabame maqaa kee dhoksuu danda'u dabalatee odeeffannoo addaa hin qabaatu. Yeroo barbaaddetti qo'annicha irratti hirmaachuu dhiisuu ykn qo'annicha keessaa ba'uu dhiisuuf mirga qabda. Akkasumas of ilaalchisee waan deebii kennuu hinbarbaanne himuuf hindirqamtu. Gaaffii yoo qabaattan teessoo armaan gadii irratti namariisissuu dandeessu:

Geetuu Sileeshii Abdiisaa (PI)

Kutaa Saayinsii Laaboraatoorii Meedikaalaa, Kolleejjii Saayinsii Fayyaa,

Yuunivarsiitii Wallaggaa

Nekemte, Itoophiyaa.

Imeelii: filenbarga2@gmail.com

Bilbila: +251917835430

Mallattoo: _____

Guyyaa: _____

5- Unka hayyamaa

Ani kan armaan gaditti mallatteesse odeeffannoo kenname dubbiseera. Qorannoon kun balaa fayyaa guddaa akka naaf hingeessisne waanan hubadheef qorannoo kana irratti hirmaachuuf walii gale.

Mallattoo hirmaataa: _____.