



**SAINT PAUL'S HOSPITAL MILLENNIUM
MEDICAL COLLEGE
DEPARTMENT OF PUBLIC HEALTH**

**PERFORMANCE EVALUATION OF MEDICAL LABORATORY
PROFESSIONALS' ON MALARIA MICROSCOPY, AFAR REGIONAL STATE,
NORTH-EAST ETHIOPIA, 2017**

BY: BIRHANU LEGESE, BSc

**A THESIS SUBMITTED TO THE DEPARTMENT OF PUBLIC HEALTH, IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF PUBLIC HEALTH IN FIELD EPIDEMIOLOGY**

March, 2018

Addis Ababa, Ethiopia

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Declaration

I, the undersigned student, declare that this research project is my original work in partial fulfillment of the requirement for the Master of Public Health in Field Epidemiology training program. I also declare that it has never been presented in this or any other higher institution and that all resources and materials used in the research work have been duly acknowledged.

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This research work has been submitted for examination with my approval.

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ST PAUL’S HOSPITAL MILLENNIUM MEDICAL COLLEGE DEPARTMENT OF PUBLIC
HEALTH GRADUATE STUDIES FINAL THESIS APPROVAL FORM

As member of the Board of Examiners of the final body of work with thesis open defense, we certify that we have read and evaluated the body of work with thesis prepared by **BIRHANU LEGESE TSEGAYE** under the title “*Performance Evaluation of Medical Laboratory Professionals on Malaria Microscopy, Afar Regional State, North-East Ethiopia, 2017*” and recommencement for the degree of Master of Public Health in Field Epidemiology.

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List of Acronyms and Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
CSA	Central Statistical Agency
EDTA	Ethylenediaminetetraacetic acid
EPHI	Ethiopian Public Health Institute
FMoH	Federal Ministry of Health
HIV	Human Immunodeficiency Virus
IQC	Internal Quality Control
IRS	Indoor Residual Spray
LLINs	Long Lasting Insecticidal Nets
MSF	Me´decins Sans Frontie`res
NEQAS	National External Quality Assurance Schemes
P.F	<i>Plasmodium falciparum</i>
P.M	<i>Plasmodium malariae</i>
P.O	<i>Plasmodium ovalae</i>
P.V	<i>Plasmodium vivax</i>
PCR	Polymerase Chain Reaction
PT	Proficiency Test
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
RDT	Rapid Diagnostic Tests
REQAS	Regional External Quality Assurance Schemes
SPSS	Statistical Package for the Social Sciences
SSA	Sub Saharan Africa
WHO	World Health Organization

Abstract

Introduction: Light microscopy is a gold standard method to malaria diagnosis. The effectiveness of malaria microscopy depends on maintaining a high level of staff performance. The aim of this study is to evaluate performance of laboratory professionals working in Health Facility Laboratories of Afar Region on malaria microscopy through proficiency slides and factors influencing their performance.

Method: We implemented institution based cross sectional study. We measured the performance of each laboratory professionals with 23 panel malaria slides. Microscopists achieving $\geq 90\%$ sensitivity and $\geq 80\%$ specificity and 95% Plasmodium falciparum detection (if present) were good performer otherwise poor performer. We collected data from November 13 to December 26, 2017 and entered to Epi Info version 7 and analyzed by SPSS version 21. We equated associated factor with performance through binary logistic regression. Odds ratio, p-value and 95% confidence interval was used to test association.

Result: Of the total 119 laboratory professionals working in Afar Region 99 (83.2%) professionals participated on this research. Of the total participants only 48(48.5%) not fulfilled the criteria to diagnose malaria at periphery level. Of total 2277 distributed slides, 1683(73.9%) correctly read and of 99 low load Plasmodium Vivax species 22(22.2%) slides correctly read. The overall Sensitivity and specificity was 86.7% (Kappa=0.73). Laboratory professionals trained malaria microscopy had better performance than not trained [AOR]=3.894, CI=1.329-11.410. Laboratory professionals with good theoretical knowledge had good performance than had poor theoretical knowledge. (AOR=4.067, CI=1.327-12.469, P=0.014). Laboratory professionals with less than 2 year experience had poor performance than those have more than 5 year experience (AOR=0.124, CI=0.030-0.51 1.943).

Conclusion and recommendation: Performance of laboratory professionals working in Afar region on malaria diagnosis under light microscopy was poor and not sufficiently skilled to detect the low density parasitaemia that help to malaria elimination program. Providing training and regular competence evaluation may improve their performance.

1. Introduction

1.1. Back ground

Malaria is a disease caused by a protozoan parasite belonging to the genus *Plasmodium*, and is spread from one person to another through bite of female *Anopheles* mosquitoes. There are more than 400 different species of *Anopheles* mosquito, but only 30 are malaria vectors of major importance. Five species of *Plasmodium* can infect human beings; two of these – *P. falciparum* and *P. vivax* – pose the greatest threat. Although *P. vivax* can cause severe illness and death, *P. falciparum* is the most dangerous malaria parasite, the most prevalent on the African continent and responsible for most malaria related deaths. *P. vivax* has a wider distribution than *P. falciparum*, and predominates in many countries outside of Africa. Although *P. vivax* can occur in Africa, the high frequency of a genetic trait known as Duffy negativity among African populations makes many individuals resistant to infection with *P. vivax* (1).

Laboratories play a significant role in the diagnosis, monitoring and treatment of malaria. As multi-drug resistant *falciparum* malaria continues to emerge and new regimens are developed for differential treatment of malaria species, accurate species detection becomes critical (2).

Malaria can be diagnosed by clinical sign and symptom, light microscopy, Polymerase chain reaction and other methods (3). The detection of malaria parasites by light microscopy thick/thin blood film remains the reference method (Gold standard) for diagnosis of malaria. Microscopy requires trained staff, well-maintained equipment, a regular supply of reliable reagents, clean water and electricity, and a well-executed quality management system. Where the training of microscopists, equipment and reagents are substandard, the microscopy services are almost always of poor quality (4).

To minimize errors and to produce reliable patient results WHO recommended Quality Assurance program for every laboratory. External Quality assessment is one part of QA program which can be applied in three main ways, each with advantages and disadvantages: i) Proficiency test (PT), where an external provider sends samples of undisclosed results to laboratories or individual testers and provides feedback on results. It is usually measure performance individual laboratory professionals; ii) Rechecking or retesting samples in higher level or peer laboratories

(inter-laboratory comparison); iii) On-site assessment by approved evaluators. On this study we implemented PT to evaluate the performance of laboratory professionals working at peripheral level.

1.2. Statement of the Problem

Nearly half of the world's population, 3.3 billion, are at risk of malaria, and 1.2 billion are leaving at high risk areas (5). Within fifteen years, 2001 and 2015, 1.3 billion malaria cases and 6.8 million malaria deaths occurred globally. Of all deaths about 6.6 million (97%) were children aged under 5 years (1). Each year, 212 million cases and 429,000 deaths of malaria occurred globally and most deaths (92%) estimated to have occurred in African (6). About 292,00 under 5 children died (6) and 1.5 million pregnant women suffered from malaria and its adverse consequences in Sub-Saharan Africa (SSA) (7). Nigeria and the Democratic Republic of the Congo contribute 35% for deaths from malaria (8).

Malaria is a major public health problem in Ethiopia and approximately 60% of Ethiopians live in malaria risk areas, and 75% of the country's landmass is favorable for malaria transmission, with malaria primarily associated with altitude and rainfall (9). The transmission peaks bi-annually from September to December and April to May following rains of the season (10). Federal Ministry of Health reported malaria was the tenth causes of outpatient morbidity and admission accounting 3.37% and 1.38% respectively in 2016. In the same year 5.17% outpatient visits, 3.71% admission and 1.16% of deaths among under five children were due to malaria (11).

Malaria is also the leading cause of morbidity and mortality in Afar Region. The transmission in the region is generally unstable, with perennial transmission in areas along the Awash River Valley specially (12). Federal ministry of health reported that total malaria case of Afar Regional State was 119,053 which contributed 5.5% to national malaria cases in 2015. In this year the incidence of malaria was 6,387 cases per 100,000 populations at risk with CFR of 0.01% (13).

To prevent complicated malaria, treatment must begin as soon as possible, generally within 24 hours after symptom onset. Effective malaria treatment requires improved diagnosis of malaria

(3,18). Malaria can be diagnosed through Giemsa-stained light microscopy, Antigen detection (RDT), Molecular methods (DNA PCR), Quantitative Buffy Coat, Dark-field microscopy, Automated blood cell analyzers (15,16).

Reliable laboratory testing remains a challenging issue in many resource-limited countries (17,18). The main source of unreliability of results includes; limited resource of laboratories, inadequate infrastructure, poorly-trained professionals and lack of standardization (19). Other obstacles that contributed to low quality are low access to financial service (20), the cultural beliefs of the patients, attrition of healthcare workers, physicians' attitudes and high work load of microscopists (4). These challenges faced in laboratories of developing countries are the main obstacles for early detection, diagnosis treatment, control and prevention of malaria (21).

Human error is inevitable however it can be minimized through designing safer systems. A study on British Medical Journal (BMJ) indicated that in the United States of America medical error was the third leading cause of death in 2013 (22). The common causes of medical errors listed on other BMJ were diagnosis error 26%, improper performance (14%), failure to supervise or monitor case (11%), failure to recognize a complication of treatment (3%) and medication error (3%) (23). In laboratory errors can be occurred in the Pre- analytical, Analytical or Post-analytical stages. The distribution of errors estimated that 46%-68.2 in the pre-analytic phase, 7-13% in the analytic phase, and 18.5-47% in the post-analytic phase (24). Overall, it is estimated that laboratory results are misleadingly wrong in 2-4% of cases (23) and had an effect 24.4%-30% on patient care (25). From the whole laboratory estimated errors about 8% (26) with range 2.7 % to 12 % (25) of them had the potential for serious harm. Most of the errors that occur in the analytical stage are caused by laboratory personnel (27).

The trust of microscopy in diagnosing also depends on the knowledge, level of skill, judgment and motivated staff. WHO recommended that an acceptable microscopic service which expected to produce cost effective, accurate, and timely result requires a comprehensive and active quality assurance (QA) programs. Without an efficient QA program, resources spent on diagnostic services are likely to be wasted and clinicians will lose confidence in the results provided by malaria microscopy (28). However most developing countries did not know the quality service of their diagnostic laboratories (29).

In Ethiopia, of 2.18 million malaria cases, 1.88 million (85.9 %) were confirmed by either microscopy or rapid diagnostic tests (RDTs) (13). However, the actual number of malaria cases in the country is estimated more than 5 million per year (9,30).

On this elimination era of malaria(31), highly competent laboratory professionals, regular external assessment, ensuring good-quality reagents and equipment are important issues (28). Performance of laboratory professionals working in Afar region is not well known even though performance of laboratory professionals done in different parts of Ethiopia (32–34). These literatures summarized that the performance of laboratory professionals in the study areas were poor. At malaria rechecking sites of Ethiopia (34) there was no malaria expert and most laboratory professionals could not have skill of malaria species identification and parasite counting. The aim of this study is to assess performance of laboratory professionals on malaria smear microscopy and factors influencing the performance of the professionals to diagnosis of malaria under light microscopy.

1.3. Significance of the study

This study provided information about the performance of laboratory professionals on malaria diagnosis under light microscope who are working in Afar region. Thereby concerned Health authorities at Federal and Regional level will have a complied data about the current performance of laboratory professionals on diagnosing malaria, able to identify identified gaps and will take appropriate actions to improve gaps. Additionally the findings of this research will provide information for policy developers at national regional organizations. Moreover, this study will also serve as an input for future performance evaluation of malaria diagnosis and other related studies and reviews.

2. Literature Review

2.1. Performance of Laboratory professionals

A comprehensive assessment of the malaria microscopy system in Indonesia, 547 microscopists participated in proficiency testing. Nearly all microscopists (413 or 95.6 %) scored at basic or in-training level, while 10 (2.3 %) were advanced, 9 (2.1 %) were reference microscopists and no microscopists achieved expert level (35).

External quality assessment of Giemsa-stained blood film microscopy for the diagnosis of malaria and sleeping sickness in the Democratic Republic of the Congo showed that *Plasmodium falciparum* gametocytes was read without errors by 44.8% of participants, whereas 37.7% made a minor error and 17.5% made a major error. Additionally on parasite negative slide 81 % participants scored as negative while the rest (19%) reported as positive (36).

On cross sectional study conducted in clinical laboratories of Hawassa town to evaluate the performance of laboratory professionals 10 slides were distributed to each 72 laboratory professionals. Of 72 participants, 14(19.4%) correctly interpreted all ten distributed slides, and 58(80.6%) missed at least one slide. 18 (25%) of the participants reported correct results of all positive samples and 67(93.3%) of participants correctly reported all four negative slides. Based on the WHO grading system, 17(23.6%) of the participants were rated as ‘in training’, 23(31.9%) as “competent”, 14(19.4%) as “reference”, and 18(25%) as “expert” level. The overall agreement between reference WHO readers and participants in the detection of malaria parasites was 88% and in the identification of different species of malaria was 74.3%. Overall agreement between participants and the experts malaria microscopists on detection and identification was 74.3% (Kappa = 0.63) and 88% (Kappa =0.76) respectively. This study found that gender, work experience, number of slides examined per day, in-service training and supervision did not had any association for poor performance of laboratory professionals (32).

Another cross sectional study to evaluate performance of laboratory professionals in Defense health facilities of Ethiopia around Addis Ababa area a total of 12 slides six stained and six unstained slides distributed to 60 study participants. From the total participants only 5 (8.3%) interpreted all the distributed slides correctly in terms of parasite detection & species identification; and the rest 55 (91.6%), each of which, interpreted at least two slides incorrectly.

Based on WHO grading system, 40 (66.7%) of participants were at “In-training”, five (8.3%) at “Advanced”, 10 (16.7%) at “Reference”, and five (8.3%) at an “Expert level” in malaria parasite detection. On this study the overall sensitivity and specificity of the professionals in detecting malaria parasites were 65.7% and 100%, respectively. The overall agreement of malaria parasite between the study subjects and expert reader was 71.4% (Kappa: 0.4) which is ‘fair agreement’. The overall agreement on identification of malaria species was 51.1% (kappa: 0.04) which is ‘slight agreement’. This research founded that laboratory professionals had difficulties in the identification of low density parasite and mixed infections. The low performance of laboratory professionals did not have association with educational level, work experience and malaria microscopy/QA training (33).

Another cross sectional study conducted in 2015 to evaluate performance of laboratory professionals working at rechecking sites had participant of 107. The investigator distributed 12 slides to each laboratory. On this finding the mean age of participants was 30 ± 5 yrs and 90 (84.1%) were men. From a total of 1070 blood film slides that were distributed for detection, 1036 (96.8%) were correctly reported for the presence or absence of malaria parasites and from a total of 856 positive blood film slides only 486 (56.8%) were reported correctly in terms of species identification. From the total participants 72.9% (78) were ‘in training’ with respect to species identification. The overall agreement on detection of malaria parasites was 96.8% (Kappa = 0.9), which is ‘perfect agreement’ while on identification of malaria species it was 64.8% (Kappa = 0.33) which is a ‘fair agreement’. Out of 107 participants, 97 (90.7%) scored <80% overall and the least accurately identified malaria parasite species was *P. malariae* (3/107; 2.8%) followed by *P. ovale* (35/107; 32.7%) (34). On this study laboratory professionals who had malaria microscopy and QA training had high performance than not training and work experience did not had any association with performance of the participants (34).

2.2. Associated factors with Performance of Laboratory Professionals

A. Training

Laboratory errors are common and that they are facilitated by defects in the equipment and mistakes by the laboratory staffs. Many of the errors are as a result of adequate training and

shortage of laboratory staff members. A comparison of literature shows that training of laboratory staffs has a direct bearing on the reduction of errors in the laboratories (27)

Refresher malaria training in four districts of Uganda indicated that pre-training test scores of participants were generally low, with an average score of 41% and post training test was improved significantly with an average of 75% score with p-value of ($P < 0.0001$) (37).

A seven day malaria microscopy training of laboratory professionals in Nigeria additionally indicated that the trainee knowledge and microscopic skills significantly increased. The mean slide reading detection, species and counting agreements in pre-training assessment were 48.9%, 27.9% and 0%, respectively, and in post-training 56.8%, 39.2% and 25%, respectively ($P < 0.001$) (38).

On research conducted among laboratory professionals working in rechecking sites of Ethiopia to evaluate their performance indicated that laboratory professionals who had malaria microscopy and QA training had high performance than not trained (Chi-Square 24.0 and $P < 0.001$) and work experience did not have any association with performance of the participants (Chi-Square 0.4 and $P < 0.823$) (34).

B. Low density parasitemia

A research conducted in Cambodia and Indonesia found that malaria reference readers (experts) had difficulty in identifying low density parasitemia. On this study only 85% of slides correctly read with densities < 100 parasites/ μ l and those slides with densities > 350 parasites/ μ l 100% correctly read (2). Researches done in Ethiopia, Hawassa town (32), Addis Ababa (33) and Malaria rechecking sites of Ethiopia (34), also agreed that laboratory professionals were challenged by slides with low parasitemia.

C. Work experience

On cross sectional study conducted in the Peruvian Amazon 68 microscopists without experience (< 1 year experience) and 76 microscopists with experience (> 1 year experience) recruited in the study. Microscopists with expertise (68.6%) had more agreement than those without expertise (48.2%). The study found that 80.2% of microscopists without experience were “in training”

while 47.7% microscopists with experience were “in training”. In the study only 1 (1.5%) microscopists were categorized in “experts” while 9 (11.8%) were categorized in “experts”. The competency assessment was acceptable (competent, referent, or experts levels) in 11.8% of the microscopists without expertise and in 52.6% from those with expertise (39).

A retrospective data review of existing PT in USA laboratory professionals with 2 years or less of experience had almost twice the odds (adjusted odds ratio, 1.849, $P=0.043$) of producing an error compared with personnel with more than 20 years of experience (40). However different researches conducted in Hawassa town (32), Addis Ababa (33) and Malaria rechecking sites of Ethiopia (34) reported that work experience did not have significant association with microscopists performance.

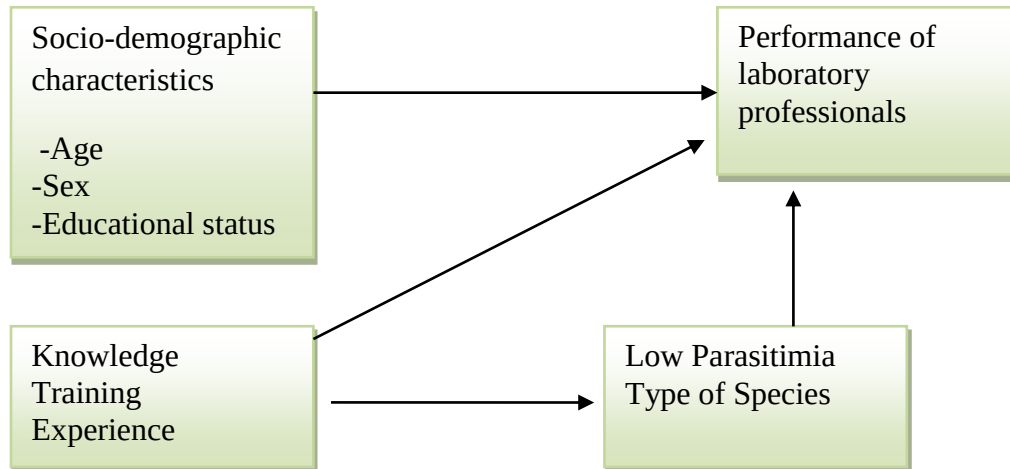
Participation of External Quality Assessment

A cohort study by Me´decins Sans Frontie`res (MSF) Operational Center Amsterdam indicated that QC program played a significant role by providing key information to conduct supervision and training for laboratory professionals. The study was conducted in 2005 with two cohorts and 2008 also two cohorts. Marked progressive improvement in the overall malaria microscopy QC performance was seen over the period (2005 and 2008). At the commencement of the QC program for the period May– December 2005 32.3% (10/31), 17.4% (4/23), and 58.1% (18/ 31) of laboratories complied with the percent agreement, FP, and FN targets, respectively. By 2008, for the period January–August there were significant improvements ($P<0.001$) in the proportion of laboratories meeting each QC target, with the results of 95.7% (45/ 47), 86.7% (13/15), and 91.3% (42/46), respectively (41).

2.3. Conceptual Framework

The performance of laboratory professionals may vary with socio-demographic characteristics like age and sex. Low knowledge of characteristics of malaria parasite at analytical stages, lack of training, inexperienced laboratory professionals decrease the performance. Increasing level of knowledge through training and experience will increase performance of laboratory professionals. Low parasitemia and species identification are difficult to Laboratory Professionals with low knowledge, less experience and without training.

Figure 1: Conceptual frame work for Performance Evaluation of Laboratory Professionals through proficiency test slides (Developed from literatures)



3. Objective

3.1. General objective

- To evaluate performance of laboratory professionals working in Health Facility Laboratories of Afar Regional State through proficiency test slides to diagnose malaria under light microscope and associated factors with performance laboratory professionals to diagnose malaria in 2017

3.2. Specific objectives

- To determine the performance of laboratory professionals in detecting malaria parasite on under light microscope.
- To determine the performance of laboratory professionals in identifying negative blood films as negative under light microscope.
- To identify associated factors influencing the performance laboratory professionals to detect and identify malaria parasite.

4. Method and Materials

4.1. Study area

The study was conducted in Afar region, North-East Ethiopia. The total geographical area is about 270,000km² located between 39° 34' and 42° 28' east longitude and 8° 49' and 14° 30' north latitude. It is generally low land having an altitude ranging from 200 meters below sea level to 1500 meters above sea level. Malaria transmission of the Afar depends on seasonal rivers in the area which is called "Aridity". Some areas of the region also affected by malaria through seasonal rain(10).

According to the regional administration office, the region has 5 zones; 32 woredas, 2 administrative towns and 404 kebeles. The mainstay of the economy is livestock husbandry. Around 70% engaged in animal rearing, 27% are agro-pastoralist and only 0.4% in farming. Based on the figures from the Central Statistical Agency projection of 2007, the region had an estimated total population of 1.89 million of whom 86.6 % are rural dwellers in 2016.

Based on the region health office report there are six public hospitals and one private maternal hospital, 77 health centres, 1 regional laboratory, 314 health posts and 29 private lower and medium clinics delivering health services for the people living in the region. Afar Regional Health Research and Laboratory Service (Afar Regional Laboratory) supports health facilities to increase quality of malaria diagnosis and other tests. In 2016/17 the laboratory collected and rechecked 3740 malaria slides (Blood Films) from 45 health facilities to check quality of laboratories.

4.2. Study Design

Institution based cross sectional study was conducted

4.3. Study Period

The data of this study was collected from November 13, 2017 to December 26, 2017.

4.4. Source Population and Study Population

All laboratory professionals working in Afar Region were source and study population of this study.

4.5. Inclusion and exclusion criteria

Inclusion criteria: Laboratory professionals working in Afar Regional State health facilities and participate on diagnosing malaria with light microscope

Exclusion criteria: Laboratory Professionals Laboratory Professionals with sick and unable to diagnose and color blinded microscopists were excluded from the study.

4.6. Sample Size

Calculation of samples size is based on single population proportion

$$n = \frac{\left(\frac{Z_{\alpha}}{2}\right)^2}{d^2} \cdot \rho q$$

Where

Z_{α} =level of confidence (Z score) (95%)

n = number of laboratory professionals to be participated in the study

p = proportion of laboratory professionals score greater than 80% performance (performance of laboratory professionals in Hawassa was 44% (32))

$q = 1 - p$

d = margin of error (0.05)

$$n = \frac{(1.96)^2}{0.05^2} \times 0.44 \times 0.56 = 378$$

Since total number of laboratory in the region is only 1 all laboratory professionals working in the region included in the study.

4.7. Sampling technique

Since all laboratory professionals working in Afar Region used as study population no need of special sampling technique.

4.8. Data collection

The data was collected through proficiency test (PT) malaria slides and questionnaires

4.8.1. Preparation of blood films for PT

Two expert microscopists who have been pre-qualified and certified by WHO as Level I Expert Microscopists (28) prepare and validate the PT slides at Adama Malaria Control Laboratory. 3-5 milliliters of EDTA anti coagulated whole blood sample was collected from patients who have typical malaria sign and symptom attend the Adama Malaria Control Laboratory after obtaining informed consent and the negative sample was collected from a person who never had history of malaria. Totally 6 different samples were collected to obtain high load P.F, medium load P.F, low load PF, high load P.V, mixed infection of P.F/P.V and negative slides. All samples were processed within one hour of collection to preserve leukocyte and parasite morphology.

High-quality microscope slides were used for staining the blood films. These slides were frosted end for labeling, free from grease, moisture or fungus. The slides were cleaned and stored before use which prevent most of the artifacts, avoid detachment and washing away of thick blood films during staining (28). From the collected blood samples, multiple set of high quality blood film PT were prepared containing both thin (with 2 μ l of blood) and thick (with 6 μ l of blood) on a single slide. After blood collection, ten negative, five P.F slides with density of 62,000 parasites/ μ L, five P.F slides with density of 1350 parasites/ μ L, one P.V slide with a density of 46,000 parasites/ μ L, one P.V slide with a density of 760 parasites/ μ L and one mixed slide with density of 88,000 parasites/ μ L. Totally 23 blood films were prepared to each laboratory professional to obtain high load P.F, high load P.V, medium load PF, low load P.V and mixed infection of P.F/P.V and negative slides.

Determining of parasite density/ μl is performed from thick film by counting asexual parasites per 200 leukocytes by assuming a standard WBC count of 8,000 leukocytes/ μl . The true counts were determined by the two malaria expert readers. Parasite counts were scored as acceptable if they were within 25% of the true count.

$$\text{Parasite}/\mu\text{l} = \frac{\text{Number of asexual parasites} \times 8000 \text{ leukocytes}}{200 \text{ leukocytes}}$$

4.8.2. Questionnaire

The questionnaires were developed from WHO and national malaria guide lines which contain general information of the laboratory on malaria, QA activity of the laboratory on malaria, socio-demographic information of the respondents and knowledge questions. The knowledge of laboratory professionals was collected through standardized pre tested questionnaire that would assess a practical skill to perform, interpret or prepare, stain and examine malaria parasite. The malaria identification performance of laboratory professionals were measured by standardized PT slides. The result of PT slides were registered on pre tested format and collected immediately after its completion. The data was collected by four laboratory professionals who first trained on the collection method of this research.

4.9. Data Quality Assurance

The questionnaires were prepared as clear and understandable way and these questionnaires were pre tested before actual data collection. The pretest was conducted among 10 laboratory professionals work in Adam town. The data collectors were trained for 2 days on how they would collect the data. They made role play practice on the training section. The supervisor and data collectors were selected from Afar Regional Health Research and Laboratory Service who have experience of supervision and data collection. Supervision was done during data collection and data was double entered. The quality of the blood film slides were properly checked against WHO standard before providing to laboratory professionals for reading and the results was critically seen for completeness. The decrease influence of equipment to performance the data collectors checked the functionality of microscope though control slide.

4.10. List of Variables

Dependent Variable: Performance of laboratory professionals on malaria microscopy

Independent Variables: Age, Gender, Educational Level, Work Experience, Training, and Knowledge

4.11. Operational definitions

Agreement: It is a combination of sensitivity and specificity that describes the number of correct answers given or the amount of covenant between expert readers and the participant's answers, so both true negatives and true positives are counted toward this measurement

Diagnostic accuracy: The proportion of both negative and positive slides correctly read. (Calculated by dividing the number of slides with correct diagnosis by the total slides examined)

Error rate: The proportion of slides incorrectly diagnosed

Knowledge: On this contexts knowledge is defined as; theoretical knowledge of laboratory professionals to examine malaria parasite, interpret and report through thin/thick blood film Gaimsa stain. Laboratory professionals score 15(75%) out of 20 question are knowledgeable and called “have good knowledge” otherwise “poor knowledge”. The cut point was decided by malaria experts in EPHI.

External quality assessment: Set of activities organized outside a laboratory or health facility (by external supervisors or an external quality provider) to effectively and systematically monitors work carried out, including not only proficiency testing but also validation of the results of routine blood slide and on-site supervision.

Performance: The skill of a Malaria Microscopists for performing an accurate examination and reporting of a malaria blood film.

Performance of laboratory professionals at Periphery level

WHO recommended that malaria microscopists achieving $\geq 90\%$ sensitivity, $\geq 80\%$ specificity and 95% *plasmodium falciparum* detection if present is achievable and

satisfactory for a peripheral-level microscopists during performance evaluation or training (28).

Good Performer Laboratory Professional: Laboratory professional has “sensitivity $\geq 90\%$ and “specificity $\geq 80\%$ ” and “ $\geq 95\%$ detection *Plasmodium falciparum* if present” is “ Good performer” or fulfill minimum WHO criteria at periphery level to diagnose malaria microscopically.

Poor Performer Laboratory Professional: Laboratory professional has “sensitivity $< 90\%$ ” or “specificity $< 80\%$ ” or “ $< 95\%$ detection *Plasmodium falciparum* if present” is “Poor Performer” or not fulfill minimum WHO criteria at periphery level to diagnose malaria microscopically.

Performance of laboratory professionals (malaria microscopists) at international level

In training : Laboratory professionals considered as in training when they score less than 70% on parasite detection, species identification and with 25% true count;

Advanced: Laboratory professionals considered as Advanced when they score greater than or equal to 70% but less than 80; on parasite detection, species identification and with 25% true count the agreement was

Reference: Laboratory professionals considered as reference when they score greater than or equal to 80% but less 90% on parasite detection, species identification and with 25% true count

Expert: Laboratory professionals considered as Expert when they greater than or equal to 90% on parasite detection, species identification and with 25% true (28). Sensitivity: Proportion of positive slides correctly read as positive

Species accuracy: The proportion of positive slides correctly identified with their species

Specificity: Proportion of negative slides correctly read as negative.

Training: On job malaria microscopy and quality assurance training of laboratory professionals for at least one week.

True count: In this context true count expressed as, average parasite density/ μl calculated by WHO malaria experts by counting asexual parasites per 200 leukocytes, by assuming a standard WBC count of 8,000 leukocytes/ μl .

4.12. Data Analysis

The data was checked for its completeness, inconsistencies and missing values and entered in Epi Info 7 then was analyzed using SPSS 21. Diagnostic performance indicators such as; Sensitivity, specificity, diagnosis accuracy (agreement), species accuracy, error rate and parasitaemia counting were calculated and compared with independent variables. Associated factors were equated binary logistic regression and odds ratio with 95% confidence interval, p-value and was used to test association.

4.13. Ethical issues

This study was conducted after approval of Institutional Review Board of SPHMMC and official letter written to Afar Regional Health Bureau. Afar Regional Health Bureau also wrote support letter to the participating facilities. Consents also asked from participating facilities and participating laboratory professionals thorough formats and signed if agreed. Information obtained about the laboratory professionals from the questionnaires, data capturing formats and the slides were kept totally anonymous. Participants had the right not to participate or to withdraw from the study at any time and also have the right to jump a question not willing to answer. The consent of those gave blood were asked to prepare blood films. The blood was tested for any other tests except malaria and those gave people gave blood had the right not to participate in the study and they have the right to withdraw at any time from the study.

4.14. Dissemination of findings

The study will be submitted to St. Paul's Hospital Millennium Medical College, Public Health Department and will serve as a reference in the library. Copy of this material will be given to Afar Regional State Health Bureau, FMoH, and other concerning bodies. This result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in related workshops and seminars.

5. Results

5.1. Socio-demographic characteristics of the participants

Of the total 119 medical laboratory professionals working in Afar Region 99 (83.2%) professionals participated on this research. The rest 18 were not present during data collection and 2 professionals were not volunteer to participate on the study. They were from 21 Woredas and 2 city administrations. They were also from 5 public hospital, 33 public health centers, 4 Tendaho sugar factory health centers and 13 private clinics. The median age of the participants was of 27 years (IQR=25-30) and 67(67.7%) male.

Table 1 Socio-Demographic characteristic of laboratory professionals working on malaria microscopy, Afar Regional State, North East Ethiopia, 2017

Variables		Frequency (Number of participants) (N=99)	Percent
Sex	M	67	67.7
	F	32	32.3
Age	20-24	21	21.2
	25-30	59	59.6
	31-35	8	8.1
	>=35	11	11.1
Educational status	Diploma	79	79.8
	First Degree and above	20	20.2
Working facility of participant	Private clinic	13	13.1
	Public Hospital	29	29.3
	Health center	57	57.6
Work experience (year)	<2	24	24.2
	2-4	28	28.3
	≥5	47	47.5
Malaria Training	yes	66	66.7
	No	33	33.3

5.1.1. Assessment of Malaria Quality assurance

Laboratory professionals done malaria IQC regularly were 84(84.8). No laboratory professional perform only thin blood film. However 14(14.1) of them perform only thick blood film. All participants, except one professional, participated on Regional External Quality Assurance Schemes. Two microscopes were excluded from the study because they were not functional or not pass by the control slides.

Table 2 Quality assurance of malaria microscopy among laboratory professionals working in Afar Regional State, North East Ethiopia, 2017

Variables		Frequency (Number of participants)	Percent
Perform Malaria IQC	yes	84	84.8
	No	15	15.2
Blood films they routinely perform	Only thin BF	0	0
	Only thick	14	14.1
	Both thin and thick BF	85	82.9
Participate REQAS/ IEQAS	Yes	98	99
	No	1	1

5.2. Performance of laboratory professionals on Malaria microscopy

Nearly half 48(48.5%) participants did not fulfill WHO minimum criteria (had poor performance) to diagnose malaria at periphery level. Of the total 2277 distributed slides, 1683 (73.9%) slides were correctly read, 132 false positive out of 990 (13.3%), 172 (13.4%) false negative out of 1287 and 290 (22.5%) species miss identification (SMI) out of 1287 slides. From 495 high load Plasmodium Falciparum 377(76.2%) slides correctly read, 44(8.9%) false negative and 74(14.9%) incorrectly read as another species (SMI). Of 99 distributed low load Plasmodium Vivax species 22(22.2%) slides correctly read, 61(61.6%) false negative and 16(16.2%) incorrectly read as other species (SMI). The overall agreement between WHO malaria experts Participants was 86.44%. The agreement of species identification accuracy was 64.1%. 86.44%, agreement in species identification accuracy was 64.1% (described on table 3 and 4). The error rate of the participants was 26.1%.

Table 3: Overall agreement of WHO malaria expert malaria slides reading and Laboratory professionals malaria slides, Afar Region North East Ethiopia, 2017

Parasite detection		WHO malaria expert's reading		Sensitivity	Specificity	Agreement	Kappa
		Positive	Negative				
Participant's reading	Positive	1115	132	86.66%	86.67%	86.44%	0.73
	Negative	172	858				
	Total	1287	990				

Table 4: Species identification accuracy of WHO malaria expert malaria slides reading and laboratory professionals malaria slides, Afar Region North East Ethiopia, 2017

Species identification		WHO malaria expert's reading			Sensitivity	Specificity	Agreement	Kappa
		P.F	P.V	Mixed				
Participant's reading	P.F	695	23	41	86.66%	NA	61.44%	0.30
	P.V	76	95	17				
	Mixed	127	5	35				
	Negative	91	75	6				
	P.O	1	0	0				
Total		990	198	99				

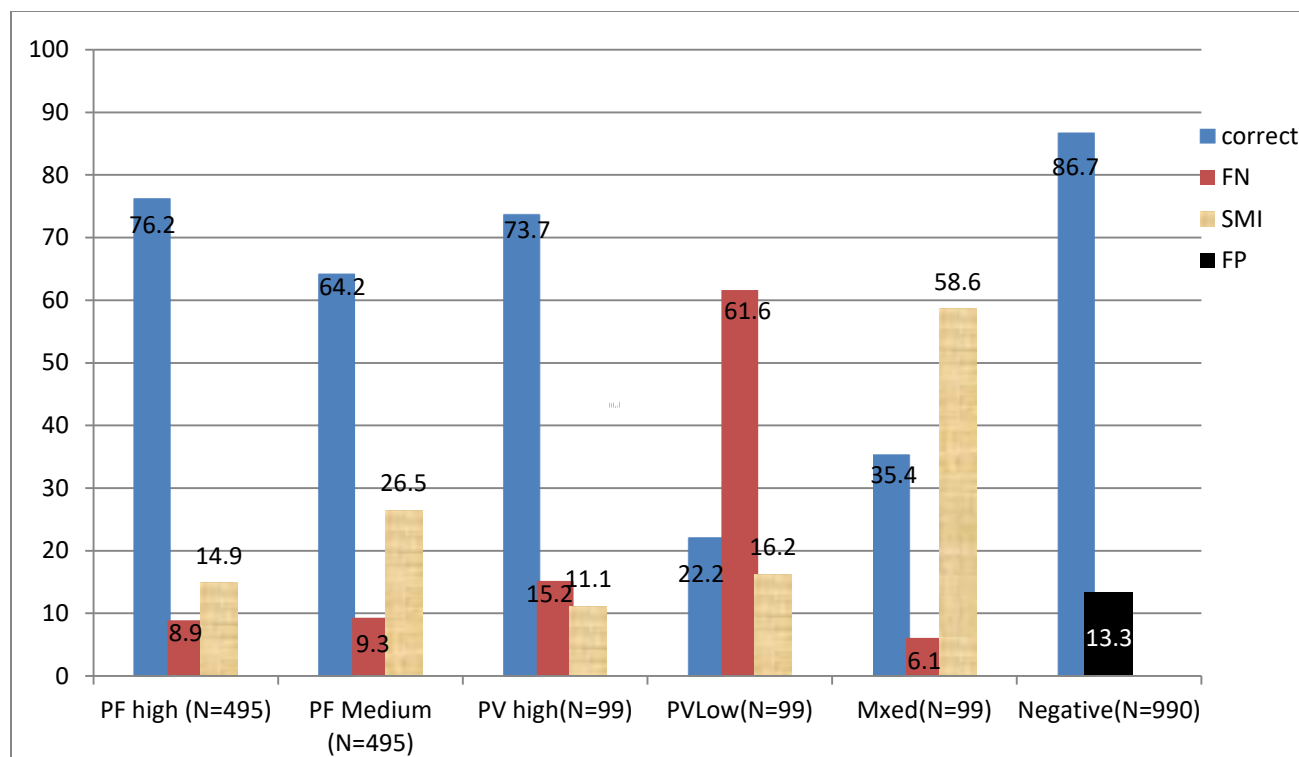


Figure 2 Performance of laboratory professionals on malaria parasite detection species identification, Afar Regional state, Ethiopia, 2017

According to WHO competence level of malaria microscopists, on parasite detection, 18(18.2%) level-I (Expert) and 16 (16.2%) level-IV (In training). The competence level (performance) of laboratory professionals on species identification showed only 14(14.1%) of them were categorized in level-I (Expert) and 16(16.2%) of them were in level-IV (In training). However, with regard to parasite quantification, no laboratory professional was categorized in level-I (Expert) because all of them did not quantify the positive slides correctly according to the standard. Therefore, all laboratory professionals participated to this research were categorized under level-IV (In training).

Table 5: Performance level of laboratory professionals on malaria microscopy, Afar Regional State, Ethiopia, 2017

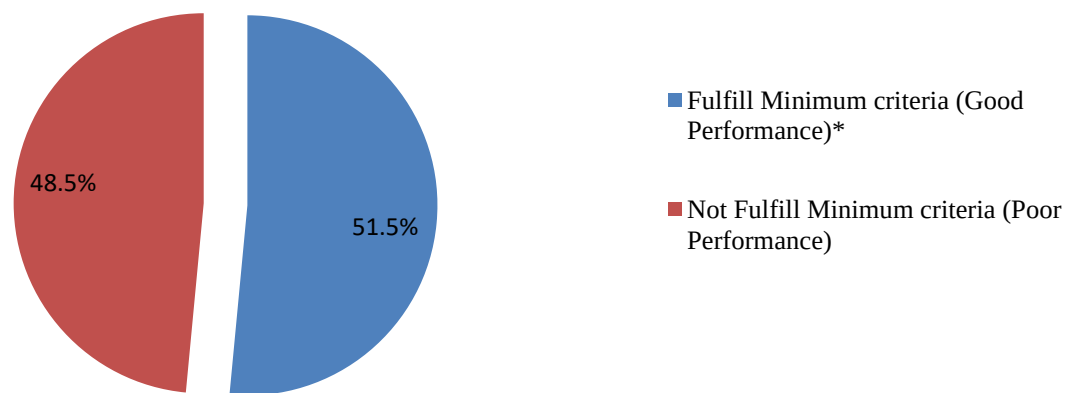
WHO competence level (Performance)		Frequency	Percent	Cumulative Percent
WHO parasite detection level (%)	90-100 (Level-I)	69	69.7	69.7
	80-89 (Level-II)	10	10.1	79.8
	70-79 (Level-III)	4	4.0	83.8
	0-69 (Level-IV)	16	16.2	100.0
WHO Species Identification level (%)	90-100 (Level-I)	14	14.1	14.1
	80-89 (Level-II)	22	22.2	36.4
	70-79 (Level-III)	14	14.1	50.5
	0-69 (Level-IV)	49	49.5	100.0
WHO Parasite Quantification level (%)	90-100 (Level-I)	0	0	0
	80-89 (Level-II)	0	0	0
	70-79 (Level-III)	0	0	0
	0-69 (Level-IV)	99	100	100

Based on WHO recommendation professionals working at periphery level, 51(51.5%) fulfilled minimum competence level (had good performance) to diagnose malaria microscopy at periphery-level. Of the total participants 72(72.7%) good on parasite detection, 81 (81.6%) parasite species identification and 51(51.6%) of them detected 95% Plasmodium Falciparum. From 99 participants only 3(3.00%) of them correctly read all slides without any errors, 16(16.2%) missed only one slide and the other participants (80, 80.8%) missed at least 2 slides and above.

Table 6 Overall Performance of laboratory professionals on Sensitivity, specificity and P.F detection, Afar Regional state, North East Ethiopia, 2017

Performance indicators		Frequency (N=99)	Percent
Sensitivity (Parasite detection)	≥ 90	72	72.7
	< 90	27	27.3
Specificity (Negative slides correctly read)	≥ 80	81	81.8
	< 80	18	18.2
Plasmodium Falciparum detection	≥ 95	51	51.5
	< 95	48	48.5

Of the total participants 51(51.5%) fulfilled World Health Organization minimum criteria of good performer in the diagnosis of malaria microscopically at periphery level.



*Fulfill Minimum criteria (Good Performance) sensitivity ≥ 90 , specificity ≥ 80 and 95% PF detection

Figure 3 Performance of laboratory professionals working on malaria microscopy, Afar Regional State North East Ethiopia, 2017

5.3. Associated factors of Performance

Bivariate analysis indicated that educational status, work experience, malaria microscopy training, performing malaria IQC regularly and theoretical knowledge had an association with performance of laboratory professionals to diagnose malaria microscopically. Other variable like age sex and place of did not have any association. Diploma holder laboratory professionals had poor performance than degree holder laboratory professionals Crude Odds Ratio (COR) =0.307 (Confidence Interval [CI] =0.101-0.934, P=0.038 Laboratory professionals had malaria microscopy training had better performance than not have malaria microscopy training. COR 4.845 (CI=1.981-11.853, P=0.001). The detail of bivariate analysis is described on table 9.

Table 7 Bivariate analysis of associated factors to performance of laboratory professionals on malaria microscopy, Afar Region, North East Ethiopia, 2017

Variables		Total Participants (N=99)		P value	COR	95% CI for OR
		Good performer(%)	Poor performer(%)			
Age group (Year)	20-24	8(8.1)	13(13.1)	0.117	0.359	0.100-1.249
	25-30	31(31.3)	28(28.3)	0.420	0.646	0.535-4.483
	≥31	12(12.1)	7(7.1)			
Sex	M	38(38.4)	29(29.3)	0.136	1.915	0.814-4.504
	F	13(13.1)	19(19.2)			
Educational status*	Diploma	37(37.4)	43(43.4)	0.038	0.307	0.101-0.934
	First degree and above	14(14.1)	5(5.1)			
Work experience (Year)*	less than 2 years	4(4.0)	20(24.2)	0.001	0.113	0.033-0.387
	2-4 years	17(17.2)	11(11.1)	0.787	0.876	0.334-2.296
	≥5 years	30(30.3)	17(17.2)			
Malaria microscopy training*	Yes	41(41.4)	22(22.2)	0.001	4.845	1.981-11.853
	No	10(10.1)	26(26.3)			
Perform malaria IQC regular*	Yes	47(47.5)	37(37.4)	0.045	3.493	1.028-11.865
	No	4(4.0)	11(11.1)			
Theoretical knowledge*	Good	24(24.2)	7(7.1)	0.001	5.206	1.970-13.762
	Poor	27(27.73)	41(41.4)			
Place of work	Private clinic	5(5.1)	8(8.1)	0.859	0.885	0.232-3.380
	Health centers	34(34.3)	23(23.2)	0.111	2.094	0.844-5.197
	Hospitals	12(12.1)	17(17.2)			

*=there is an association, CI= confidence interval, COR= Crude Odds Ratio

Multivariable logistic regression analysis showed that malaria microscopy on job training, work experience and theoretical knowledge had association with performance of laboratory professionals to diagnose malaria. Laboratory professionals had malaria microscopy training had better performance than not have malaria microscopy training (Adjusted Odds Ratio [AOR]= 3.894 CI=1.329-11.410). This means laboratory professional trained malaria microscopy had 3.89 times performance than not trained malaria microscopy. Laboratory professionals answered provided theoretical knowledge 75% and more had good performance than those not answered less than 75% of provided questions. (AOR=4.067, 1.327-12.469 CI=P=0.014). Laboratory professional answered 75% or more provided questions had 4.07 times performance than those answered less than 75%. Additionally those participants worked less than 2 year had poor performance than those have more than 5 year experience (AOR=0.124, CI= 0.030-0.51 1.943). Laboratory professionals with less than 2 year experience had 8.073 times probability to be poor performer than professionals those have more than 5 year experience.

Other variable which had an association on bivariate analysis such as educational status and performing malaria IQC regularly did not have any association on multivariable logistic regression. Table 10 shows the detail of multivariable logistic regression.

Table 8 Multivariable logistic regression analysis of associated factors to performance of laboratory professionals on malaria microscopy, Afar Region, North East Ethiopia, 2017

Variables		Total participants (N=99)		P value	AOR	95% CI for OR
		Good performance (%)	Poor performance (%)			
Educational status	Diploma	37(37.4)	43(43.4)	0.091	0.305	0.077-1.210
	First degree and above	14(14.1)	5(5.1)			
Work experience (Year)*	less than 2 years	4(4.0)	20(24.2)	0.004	0.124	0.030-0.515
	2-4 years	17(17.2)	11(11.1)	0.703	1.250	0.397-3.940
	≥5 years	30(30.3)	17(17.2)			
Malaria on job training*	Yes	41(41.4)	22(22.2)	0.013	3.894	1.329-11.410
	No	10(10.1)	26(26.3)			
Perform malaria IQC regular	Yes	47(47.5)	37(37.4)	0.3	2.184	0.498-9.586
	No	4(4.0)	11(11.1)			
Theoretical knowledge*	Good	24(24.2)	7(7.1)	0.014	4.067	1.327-12.469
	Poor	27(27.73)	41(41.4)			

*=there is an association, CI= confidence interval, AOR= Adjusted Odds Ratio

6. Discussion

The FMOH national malaria strategic plan (NMSP) 2014-2020 aimed that microscopy is the primary means of malaria diagnosis at hospitals and health centers, and malaria RDTs at rural health posts which helps for malaria elimination program (42). The detection of malaria parasites by light microscopy thick/thin blood film remains Gold standard for diagnosis of malaria which is needs competent laboratory professionals or malaria microscopists (4). This research is conducted to support the elimination of malaria in Ethiopia especially, Afar Region by assessing the performance of laboratory professionals. Literatures indicated that most laboratory professionals or malaria microscopists in diagnosing malaria have poor performance (32–35).

On this study, 48.5% of laboratory professionals had poor performance on diagnosing malaria microscopically. No laboratory professional categorized under Expert, Reference and Advanced Level. The overall sensitivity, specificity and species accuracy were 86.7%, 86.1% and 64.1% respectively. Their performance was associated with experience, training and theoretical knowledge.

WHO recommended that malaria microscopists achieving $\geq 90\%$ sensitivity, $\geq 80\%$ specificity and 95% plasmodium falciparum detection if present is achievable and satisfactory for a peripheral-level microscopists during training or performance evaluation (28). We also analyzed this study based on this recommendation to identify factors associated with performance of laboratory professionals. Of the total study participants 48(48.5%) of them score < 90 Sensitivity, < 80 specificity and unable to detect 95% PF.

On this study all participants (99 or 100%) were categorized under “in training” level. On research conducted in Indonesia nearly all malaria microscopists (413 or 95.6 %) categorized under “in training” level (35). Laboratory professionals working at malaria rechecking site of Ethiopia (34) majority of them (78 or 72.9%) were ‘in training’, and laboratory professionals working at Addis Ababa showed (40 or 66.6%) were ‘in training’(33). However on researches in Indonesia and Malaria rechecking sites there were reference and advanced level and no Expert level (34,35). Absence of malaria microscopy training or lack of experience (exposure) in diagnosing malaria may lid to poor performance. Poor performance of laboratory professionals in diagnosing

malaria may challenge malaria elimination agenda in Ethiopia, the community may not trust the diagnosis and clinicians not use microscopic results.

On this research the participants were categorized under “in training” level due to quantification error. None of the participant did not quantify parasite density on each of malaria positive slides using parasite per microliter against white blood cells on thick blood film. Almost all (95.6%), microscopists in Indonesia (35) and 100% in Hawasa (32) could not quantify. In routine malaria diagnosis and reporting system laboratory professionals did not quantify parasite density and they may forget how to quantify even though we told them the quantification procedure. Not quantifying the parasite may be due to lack of training, or lack of awareness of the advantage of the quantification, or it may be due to lack of commitment to adhere to specified time for counting parasite with the recommended quantification system. Quantification is used to monitor patient response to treatment

Sensitivity of the participant on this study was 86.7%. It was almost equivalent (82%) with laboratory professionals in Hawassa (32), higher (65.7. %) than laboratory professionals worked in Addis Ababa(33) and lower (96.8%) than laboratory professionals of malaria rechecking site of Ethiopia(34). Addis Ababa is low malaria risk area (10) and the professionals in the area may not have daily exposure to diagnose malaria; Afar and Hawassa is malarious area (10). So professionals in the area may easily detect malaria parasite. Professionals at rechecking site expected to have better detection than professionals at periphery level. Difficulty in detection may be due to lack of training, or may be lack of experience in diagnosing malaria, or may not scan 100 fields or may not take insufficient time for scanning. Miss identification of malaria species, specially missing of PF may cause malaria related cases.. If sensitivity of malaria diagnosis increased, communities at risk for malaria have greater confidence in their services and clinicians use the microscopy services more effectively. Additionally the patients get malaria treatment and malaria related deaths may decrease.

Specificity of laboratory professionals on this study was 86.1%. It is higher sensitivity (68.6%) than laboratory professionals in Democratic republic of Congo (43). For laboratory professionals in Democratic republic of Congo only one negative slide distributed may decrease the sensitivity. It was lower than laboratory professionals in Hawassa (96.5%) (32), than laboratory

professionals worked in Addis Ababa (100%) (33) and laboratory professionals of malaria rechecking site of Ethiopia (96.7%) (34). Difficulty in sensitivity may be due to lack of training, or may be lack of experience in diagnosing malaria, not take insufficient time for scanning, or they may not be able to differentiate artifact from malaria parasite. If true malaria negative slides read as malaria positive, community at risk may not trust the service, clinicians may not trust microscopy service, and the patients may take unnecessary treatments which may lead to drug resistant.

On this Species accuracy of laboratory professionals working in Afar was 64.1% (Kappa=0.298). Only 48.5% of the participants detected P.F correctly. It was equivalent with laboratory professionals of malaria rechecking site of Ethiopia 64.8 % (Kappa= 0.33) (34). It was lower than laboratory professionals in Hawassa 74.3% (Kappa=0.63) (32) and higher than laboratory professionals worked in Addis Ababa 51.1% (Kappa 0.04) (33). Laboratory professionals in Addis Ababa may not have exposure to different species. On malaria rechecking site of Ethiopia the four species were distributed and they couldn't identify PO and PM. Difficulty in identification may be due to lack of training, or may be lack of experience in diagnosing malaria, or may be not scanning thin blood film. Miss identification of malaria species, specially missing of PF may cause malaria related deaths.

On this study those professionals get malaria training had better performance than not trained laboratory professionals. In Indonesia the sensitivity of trained professionals better than not trained (69.1 vs 57 %, $p < 0.001$). Additionally, species accuracy of trained professionals was better than not trained (37.2 % vs 27.7 %, $p < 0.001$) (35). Similarly, trained professionals at rechecking site of Ethiopia had better performance than not trained malaria microscopy ($P < 0.001$) (34). Researches done in Uganda(37) and Nigeria(38) also that training has a power to increase performance of laboratory professionals to diagnose malaria microscopically. However among the laboratory professionals Addis Ababa surrounding area (33) and Hawassa town (32), having malaria training not associated with their performance.

Our findings also showed that those participants worked less than 2 years had poor performance than those have more than 5 year experience (AOR=8.073, $P=0.004$). In USA laboratory professionals with 2 years or less of experience had almost twice the odds (AOR, 1.849, $P=0.043$) of producing an error compared with personnel with more than 20 years of experience

(40). On a study conducted in the Peruvian Amazon 68 microscopists without experience (< 1 year experience) and 76 microscopists with experience (>1 year experience) recruited in the study. Microscopists with more than 1 year experience (68.6%) had more agreement than those with less than 1 year (48.2%) (39).. However on researches done in Ethiopia specially Hawassa (32), Addis Ababa (33) and malaria rechecking site (34) work experience and performance of laboratory professionals not associated. The variation with experience may be due exposure to diagnose malaria, or may be lack of basic malaria microscopy training. Working in laboratory needs experience and exposure to minimize analytical stage errors. Unless with support of senior microscopists and training inexperienced (“fresh”) laboratory professionals may produce false results that affects the patients, decrease trust of clinics and the community on the microscopy.

On current study laboratory professionals with good theoretical knowledge had better performance than laboratory professionals with good theoretical knowledge. The theoretical knowledge questions were pictorial and they did not contain other theoretical questions about transmission, mosquito, geographical distribution, staining and reagents. Laboratory professionals with good performance detecting and identifying malaria simply answered the questions because they can describe what they saw under microscope.

The most incorrectly slides read by the participants were low load plasmodium vivax and mixed (plasmodium falciparum and plasmodium vivax). Only 22.2% low load plasmodium vivax slides and 35.4% mixed slides correctly read. Not only professionals at periphery level but also malaria experts had difficulty in identifying low density parasitimia (2). On Researches done in Ethiopia , Hawassa town (32), Addis Ababa (33) and Malaria rechecking sites of Ethiopia (34) showed laboratory professionals missed low density parasitimia slides. This indicates the need of training with special emphasis for detection at a low level of Parasitemia and species identification. Unable to detect malaria at low density may lead to treatment error and also difficult to malaria illumination strategies which needs accurate laboratory diagnosis. The assessment identified that the laboratory professionals in Afar region microscopists are not sufficiently skilled to detect the low density parasitaemia associated with malaria elimination program.

7. Limitations

This study come with some limitations, Performance of Malaria Microscopists could not include all malaria microscopists in the study sites; only those who were available during the time of data collection were recruited. The species of malaria parasites used for malaria blood film slide panel preparation were not characterized or molecularly typed by polymerase chain reaction, only identified by certified malaria expert (WHO level-I). Only the two species, *Plasmodium falciparum* and *Plasmodium vivax*, used for this study: since the National Malaria Slide Bank not functional this avails other plasmodium species not common in Ethiopia.

8. Conclusion

On the current study the performance of laboratory professionals on detection and identification malaria diagnosis under light microscopy was poor. The most microscopists missed low density parasitemia and mixed infections which may lead to under treatment to antimalarial drugs. Almost half of the laboratory professionals missed *plasmodium falciparum* which is the most prevalent species and leading malaria related deaths. Associated factors for the poor performance of laboratory professionals were lack of training, experience and their theoretical knowledge. Professionals trained on malaria, more than 2 year experience of malaria diagnosis and those good with theoretical knowledge had good performance in diagnosing malaria microscopically. The assessment identified that the laboratory professionals in Afar region microscopists are not sufficiently skilled to detect the low density parasitaemia associated with malaria elimination program.

9. Recommendations

- To fill those gaps Afar regional health bureau and other concerned stakeholders have to give malaria training to all laboratory professionals
- Afar Regional health bureau should perform regular malaria competency assessment for laboratory professionals
- Universities or Training institutions should train their students on malaria parasite detection and species identification
- Laboratories should practice on positive blood film slides regularly to improve their skill on detection & species identification.
- Laboratory professionals should increase their knowledge by reading reference books malaria job aids
- Afar Regional health bureau should give malaria training for new employees before deployment to working area

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Annexes

Annex I: Consent form for Blood Donors (English Version)

My name is Birhanu Legese and I am MPH student in Field Epidemiology at St. Paul's Hospital Millennium Medical College. I am doing a research entitled "Performance Evaluation of Medical Laboratory Professionals on Malaria Microscopy in Afar Region". The objective of the study is to assess the Performance of Malaria Microscopists Working at Hospitals, Health Centers and Clinics.

To assess the performance I have to collect sample from different donors and I have to produce many blood film slides. So to get this I have to collect sufficient volume of blood from volunteers. If you are agree to participate in the study, about 3-5 ml of blood will be collected from you or you will allow us to use the sample that you will give for your medical examination and you will be interviewed. During collection of blood, you may feel some discomfort, but this does not produce serious pain. All the data obtained will be kept strictly confidential by using only code numbers and locking the data, only study personnel will have access to the files. The sample will be coded and the result will not be identified by names. There will be no costs to you as a result of taking part in this study and you are not asked to pay for the laboratory examination. Participating and not participating has no influence on the service you seek to get.

Participant's response: I am free to decline to be in this study, or to withdraw from it at any point and also to stop blood donation if I will feel discomfort. My decision as to whether or not to participate in this study will have no influence on my present or future medical service. My signature below indicates that I agree to participate in this study.

Signature of Person Obtaining Consent _____ date of signature_____

Annex II: Consent form for Blood Donors (Amharic Version)

ብርሀኑ ለገሰ እባላለሁ በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ በማስትርስ ዲግሪ የፊልድ ኢፒደሚዮሎጂ ተማሪ ስሆን የመመረቂያ ጥናቴን “Performance Evaluation of Medical Laboratory Professionals on Malaria Microscopy in Afar Region” በሚል ርዕስ በመስራ ላይ እገኛለሁ። የጥናቴ አላማ በአፋር ክልለ በሚገኙ ሆስፒታሎች፣ ጤና ጣቢያዎች እና ክሊኒኮች የሚሰሩትን የህክምና ላቦራቶሪ ባለሙያዎችን ወባ የመመርመር ብቃታቸውን ለመለካት ነው።

የእነዚህን የላቦራቶሪ ባለሙያዎች ብቃት ለመለካት ከተለያዩ በጎ ፈቃደኛ ሰዎች የደም ናሙናዎችን በመሰብሰብ የወባ መመርመሪያ ስላይዶችን በማዘጋጀት ላይ እገኛለሁ። እርስዎ ደም ለመስጠት ፈቃደኛ ከሆኑ ከ 3-5 ሲ.ሲ ደም ይወሰድና በደም ውስጥ የወባ አምጪ ተዋህሲያን መኖራቸውና አለመኖራቸው ይምረመራል። በጨማሪም አንዳንድ ጥያቄዎችን እንጠይቅታለን። ደሙ ከእጅዎ ላይ በሚወሰድበት ጊዜ ትንሽ ህመም ከሚወሰድበት ቦታ ላይ ህመም ሊሰማዎት ቢችልም ቶሎ ይተዎታል። ከእርስዎ የተወሰደው ደም ከወባ ምርመራ ውጪ ሌላ ምንም ምርመራ አይደረግለትም። ከእርስዎ የሚወሰዱት መረጃዎች እና የደም ናሙና ላይ የእርስዎ ስም የማይጻፍ ሲሆን መረጃዎቹ የሚያዙት በሚስጢር ቁጥሮች ይሆናል። ሁሉም መረጃዎች በሚስጢር የሚያዙ ሲሆን ለሌላ ሥስተኛ ወገንም ተላልፈው አይሰጡም። የወባ ምርመራ ውጤትዎን ለመወሰድ ምንም አይነት ክፍያ የማይከፍሉ ሲሆን በጥናቴ በመሳተፍዎ ወይም ባለመሳተፍዎ በምርመራ ውጤትዎ ላይ ምንም ለውጥ የለውም።

የተሳተፈው መልስ፡- እኔም በጥናቴ ላይ የተሳተፍኩት የጥናቴ አላማ ገብቶኝ ያለማንም አስገዳጅነት በፈቃዴ ነው። የደም ናሙናወን በምስጥበት ጊዜ ያመኛል ብዩ ከሰጋሁ ደም አለመስጠት እችላለሁ። ጥናቴን በማንኛውም ጊዜ የማቋረጥና መልስ መስጠት ለማልፈልጋቸው ጥያቄዎችን አለመመመለስ እችላለሁ። እንዲሁም በዚህ ጥናት ተሳታፊ ብሆንም በልሆንም አሁን ለሰጠሁት ደም የምርመራ ውጤትና ለወደፊት በመገኘገቸው የህክምና አገልግሎቶች ላይ ምንም ተፅእኖ እንደሌለው ተገንዟለሁ።

የፈቃደኛ ተሳታፊው ፊርማ _____

የፊርማ ቀን _____

Annex III: Consent Form for Laboratory professionals (Amharic Version)

ብርሀኑ ለገሰ እባላለሁ በቅዱስ ጳውሎስ ሆስፒታል ሚኒስቴር ሜዲካል ኮሌጅ በማስትርስ ዲግሪ የፊልድ ኢፒደሚዮሎጂ ተማሪ ስሆን የመመረቂያ ጥናቴን “Performance Evaluation of Medical Laboratory Professionals on Malaria Microscopy in Afar Region” በሚል ርዕስ በመስራ ላይ እገኛለሁ። የጥናቴ አላማ በአፋር ክልል በሚገኙ ሆስፒታሎች፣ ጤና ጣቢያዎች እና ክሊኒኮች የሚሰሩትን የህክምና ላቦራቶሪ ባለሙያዎችን ወባ የመመርመር ብቃታቸውን ለመለካት ነው።

የላቦራቶሪ ባለሙያዎች ወባ የመመርመር ብቃት ለመለካት ጥራታቸው የተረጋገጡ በፈቃደኛ ላቦራቶሪ ባለሙያዎች የሚሰሩ 23 የወባ ስላይዶችን ይዣለሁ። እርስዎም የጥናቴ ተሳታፊ እንዲሆኑ የተመርጡ ሲሆን ስላይዶቹን እንዲሰሩልኝና ጥያቄዎችን እንዲሞሉልኝ የእረስዎን ፈቃድ እጠይቃለሁ። በጥናቴ ለመሳተፍ አይገደዱም፤ እንዲሁም ጥናቴን በፈለጉት ጊዜ ማቋረጥ ይችላሉ። በጥናቴ መሳተፍዎ ወይም ባለመሳተፍዎ በስራዎ ላይ፣ በወደፊት እድገትዎ ላይ ምንም ተፅዕኖ አይኖረውም። የእርዎ መረጃ የሚያዘው በሚስጥር ሆኖ የእርስዎ ስም ሳይጻፍ በሚስጥር ቁጥር ይሆናል። መረጃዎቹም ከእርዎ ፈቃድ ውጪ ለሌላ ሶስስትኛ ወገን አይሰጡም። በመሆኑም ለመሳተፍ ፍቃደኛ ከሆኑ በመጀመሪያ በመጠየቂያው ላይ ያሉትን ጥያቄዎች ይሞላሉ ከዚያም ስላይዶቹን ይሰሩሉ። አንድ ስላይድ ለመስራት 10 ደቂቃ የተሰጠ ሲሆን በአጠቃላይ ሃያ ሦስቱን ስላይዶች ሰርቶ ለማጠናቀቅ ሦስት ሰዓት ከሃምሳ ደቂቃ ተሰጥቷል።

የተሳተፈዉ መልስ:- እኔም በጥናቴ ላይ የተሳተፍኩት የጥናቴ አላማ ገብቶኝ ያለማንም አስገዳጅነት በፈቃዴ ነው። ጥናቴን በማንኛውም ጊዜ የማቋረጥና መልስ መስጠት ለማልፈልጋቸው ጥያቄዎችን አለመመመለስ እችላለሁ። እንዲሁም በዚህ ጥናት ተሳታፊ ብሆንም በልሆንም በስራዬና ወደፊት በሚኖረኝ እድገቴ ላይ ምንም ተፅዕኖ እንደሌለው ተገንዝቢያለሁ።

Annex IV: Consent Form for Laboratory professionals (English Version)

My name is Birhanu Legese and I am MPH student in Field Epidemiology at St. Paul's Hospital Millennium Medical College. I am doing a research entitled "Performance Evaluation of Medical Laboratory Professionals on Malaria Microscopy in Afar Region". The objective of the study is to assess the performance of malaria microscopists working at Hospitals, Health Centers and Clinics.

To assess the performance of medical laboratory professionals I have 23 validated blood film slides which will be examined by volunteer participants. So you are selected to participate on this study to do malaria examination and to answer some questions related to your profession. All the data obtained will be kept strictly confidential by using only code numbers and locking the data. Only study personnel will have access to the files. The result will not be identified by names, it will be coded. If you are agreeing to participate in the study you will have 10 for each slide and totally three hours and fifty minutes allocated to examine all slides.

Participant's response: I am free to decline to be in this study, or to withdraw from it at any point and also to jump a question that feels me discomfort. My decision as to whether or not to participate in this study will have no influence on my present or future Carrier. My signature below indicates that I agree to participate in this study.

Signature of Person Obtaining Consent_____ date of signature_____

Annex V Questioner

Name of Data Collector _____ Date of data collection _____

Participant code, _____ Zone, _____ Woreda, _____ Facility type HC / Hosp. /Private

Section I: Scio-demographic information

1. Age _____
2. Sex 0. Male 1. Female
3. Educational Status 0. Diploma 1. First degree 2. Second degree and above
4. Work experience 0. < 2 years 1. 2-4 years 2. >5 years
5. Did you have on job malaria training 0. No 1. Yes
6. If yes for question 5, when did you take the training?
0. Within the last 12 months 1. 1 or 2 years ago 3. 3 years ago

Section II: Laboratory assessment For Malaria QC

7. Does your laboratory participate on malaria REQAS? (Observe documents and feedbacks)
0. Yes 1. No
8. Does your laboratory participate on malaria NEQAS or IEQAS? (Observe documents and feedbacks)
0. Yes 1. No
9. Does your laboratory perform malaria IQC? (Observe documents and feedbacks)
0. Yes 1. No
10. Which blood film do you prepare when you routinely diagnose malaria?
0. Only thin blood film 1. Only thick blood film 2. Both thin and thick blood films

Section III: Knowledge assessment

These questions assess the knowledge of laboratory professionals at analytical stage of malaria diagnosis especially when they are working on microscopy and post analytical stages. The questioner do not has per analytical stage and general malaria knowledge.

11. Which objective do you use to diagnose and report malaria parasite?

0. 10X 1. 40X 2. 100X oil immersion

12. Which type of blood film is more sensitive to detect malaria parasite?

0. Thin blood film 1. Thick blood film

13. Which type of blood film is more specific to confirm malaria species?

0. Thin blood film 1. Thick blood film

14. More than one parasites in a single red cell is common characteristics of _____ species

0. Plasmodium Falciparum
1. Plasmodium Vivax
2. Plasmodium Ovale
3. Plasmodium Malariae

15. Red stippling (Schuffner's dots) seen with _____ species

0. Plasmodium Falciparum only
1. Plasmodium Vivax and Plasmodium Ovale
2. Plasmodium Ovale only
3. Plasmodium Malariae only

16. Maurer's clefts seen with _____ species

0. Plasmodium Falciparum only
1. Plasmodium Vivax only
2. Plasmodium Ovale only
3. Plasmodium Malariae and Plasmodium Ovale

17. Which species characterize with presence of marginal or appliqué form

0. Plasmodium Falciparum
1. Plasmodium Vivax
2. Plasmodium Ovale
3. Plasmodium Malariae

18. Banana/crescent shape gametocyte is for _____ species

0. Plasmodium Falciparum
1. Plasmodium Vivax
2. Plasmodium Ovale
3. Plasmodium Malariae

19. For which species RBCs not enlarged?

- 0. Plasmodium Falciparum only
 - 1. Plasmodium Vivax only
 - 2. Plasmodium Ovale Plasmodium Vivax
 - 3. Plasmodium Falciparum and Plasmodium Malariae
 - 4. Plasmodium Malariae only
- 20.** Which species usually has double chromatin dots?
- 0. Plasmodium Falciparum
 - 1. Plasmodium Vivax
 - 2. Plasmodium Ovale
 - 3. Plasmodium Malariae
- 21.** What is the color of chromatin dot (Parasite nucleus) by Geimsa stain?
- 0. Red 1. Blue 2. Yellow-gold 3. Pink
- 22.** What is the color of the malaria parasite cytoplasm by Geimsa stain?
- 0. Red 1. Blue 2. Yellow-gold 3. Pink
- 23.** What is the color of the malaria pigment by Geimsa stain?
- 0. Red 1. Blue 2. Yellow-gold, Brown-black 3. Pink
- 24.** Enlargement of infected RBC is characteristics of _____ parasite
- 0. P/Vivax only 1. P/ falciparum only 2. P/Vivax and P/ovale 3. P/ falciparum and P/malariae
- 25.** Which spices has large, ring and amoeboid shape trophozoite?
- 0. P/Vivax 1. P/ falciparum 2. P/ovale 3. P/malariae
- 26.** How many fields do you scan a blood film to report as negative on thick blood film?
- 0. 10 fields 1. 20 fields 2. 70 fields 3. 100 fields
- 27.** How many fields do you scan a blood film to report as negative on thin blood film?
- 0. 10 fields 1. 20 fields 2. 100 fields 3. 200 fields
- 28.** Parasite quantification can be performed against _____ WBCs
- 0. 50 WBCs 1. 100 WBCs 2. 150 WBCs 3. 200 WBCs
- 29.** What is the purpose of malaria parasite quantification/ counting?
- 0. To monitor the evolution of the disease
 - 1. To see the efficacy of the therapy
 - 2. To know type of species

3. 0 and 1

30. What do you do if you see schizonts of *Plasmodium falciparum*? _____

0. Schizonts never found on blood film
1. Report as routine reporting condition
2. Immediately report to the physician
3. Schizonts are not reportable

Annex V: Performance assessment report form

(Answer sheet for malaria slides)

Questionnaire Number: _____ Code of Health Facility: _____

Interviewee Code: _____ Date of Report: _____

NB: Give √ sign for detection (for negative and positive) but write the detail for stage and parasite density.

S/ Number	Slide ID	Negative	Positive					Parasite load (Semi- quantitative)	Parasite Density(Only for requested) (quantitative)	Remark
			P.F	P.V	P.O	P.M	Mixed			
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
Total score										

Signature ----- date -----

Annex VI: Dummy tables

performance classification	percent agreement on Detection of malaria parasite	Frequency (n=___)	Present%
Expert	$\geq 90\%$		
Reference	$\geq 80\%$		
Advanced	$\geq 70\%$		
In-training	$<70\%$		
Total			

performance classification	percent agreement on identification malaria species	Frequency (n=___)	Present%
Expert	$\geq 90\%$		
Reference	$\geq 80\%$		
Advanced	$\geq 70\%$		
In-training	$<70\%$		
Total			

Participant reading		WHO expert reading		Sensitivity	Specificity	Agreement
		Positive	Negative			
(Malaria detection)	Positive					
	Negative					

