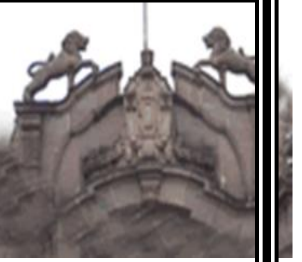

Performance Evaluation of Malaria Microscopists Working at Malaria External Quality Assessment Rechecking Laboratories in Ethiopia.

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A research Thesis submitted to the department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, School of graduate studies, Addis Ababa University. In partial fulfillment of the requirements for the degree of Master in clinical laboratory sciences, Diagnostic and Public Health Microbiology Specialty Track.

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Research Thesis Submission Form

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Full Title of the Research Project	Performance Evaluation of Malaria Microscopists Working at Malaria EQA Rechecking Laboratories in Ethiopia
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I the undersigned was conducted this research by adhering with all responsibilities for the scientific and ethical conduct of the research. I was provided a timely progress report to my advisors and got the necessary advice and approval from them in the course of the research. I was communicated timely to my advisors and all stakeholders involved in the study including any source of funding for this research.

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ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES, SCHOOL OF ALLIED HEALTH SCIENCES, DEPARTMENT OF MEDICAL LABORATORY SCIENCES

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Abbreviations

BF	Blood Film
DBS	Dried Blood Spot
EDTA	Ethylene Diamine Tetraacetic Acid
EFY	Ethiopian Fiscal Year
EPHI	Ethiopian Public Health Institute
EQA	External Quality Assessment
FMoH	Federal Ministry of Health
HCL	Health Center Laboratories
HL	Hospital Laboratories
HWH	Hydas World Health
MBFs	Malaria Blood Films
NLQS	National Laboratory Quality System
PCR	Polymerase Chain Reaction
PHCUs	Primary health care units
QA	Quality Assurance
RL	Regional Laboratory
SOP	Standard Operating Procedure
SPA	Services Provision Assessment
SRL	Sub-Regional Laboratories
USA	United States of America
WHO	World Health Organization

Operational Definitions

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

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

Donors: A person who provides specific volume of blood which is used for some activities. In this context it is a person who provides 3-5ml of blood for the preparation of blood film.

In Training: It is the lowest WHO classification on the performance of malaria microscopists. Personnel with this performance need an immediate solution to fill their gap, they have to get training, they cannot able to provide malaria microscopy services up to they get appropriate training.

Malaria Microscopist: A person who uses a microscope to read blood films to aid or confirm the diagnosis of malaria and reports on their findings. The term is used in this proposal to include personnel at all levels of a malaria programme involved in this work, from professors involved in teaching and research to village health volunteers specifically trained in malaria microscopy.

Rechecking laboratories (sites): Laboratories which collect and conduct rechecking of slide readings. It can be health center, hospital, sub-regional or regional reference laboratory.

Testing Laboratory (site): Is a laboratory which performs routine tests (those giving services directly to the patient).

Validator: It is the term stands for expert microscopists; those who were participated on confirming the result of blood film slides.

Abstract

Background: *The Performance of Malaria Microscopists in all health facilities have been raised concerns by many experts. Microscopic diagnosis of Giemsa stained thick and thin blood films by skilled microscopists has remained the standard laboratory method for the diagnosis of malaria. Microscopists who are working at Malaria Rechecking Laboratories have to be competent to cross check blood film slides which are collected from testing sites.*

Objective: *The current study aims to assess the Performance of Malaria Microscopists and Malaria EQA Rechecking Laboratories in Ethiopia from February 1-May 10, 2015.*

Methods/design:*A cross-sectional study design was conducted to assess the performance of 107 Malaria Microscopists who were working at 23 Malaria Rechecking Laboratories in Ethiopia. A set of 12 blood film slides containing Negative and positive (different species, stage, parasite density) results were distributed to each Malaria microscopists and 10 minutes were given for each blood film slides. Then all data were analyzed using SPSS Version 20 and agreement in detection and species identification of malaria parasites between participants and expert microscopists was estimated using the Kappa score. Chi Square was used for categorical data and P value (P<0.05) was considered significant.*

Result: *From a total of 107 study participants, the maximum number of participants 90 (84.1%) were a male and most of them were working at regional reference laboratory 54(50.5%).About 34(31.8%) participants were used unrecommended quantification system. Overall, the sensitivity of participants in detection and species identification of malaria parasites were 96.8% and 56.7%, respectively. The overall agreement on detection and identification of malaria species was 96.8% (Kappa = 0.9) and 64.77% (kappa = 0.33), respectively. The least malaria species which were identified correctly by the participants were P. malerea and P.ovale which was identified correctly 2.8% and 32.7%, respectively. The number of participants who were scored <80% was higher among participants with diploma 25(100%) followed by participants with degree 59(93.6%) and participants with MSc and above 13(68.4%)(P=0.001). Participants at Hospital laboratory had higher percent agreement (72.3 %, Kappa=0.51) compared with participants from other health facilities.*

Conclusion and Recommendation: *Agreement of the participants with expert microscopist in the identification of different malaria species and quantification were very low. Most participants were not identified P. malerea and P.ovale correctly. Therefore, to fill those gaps we have to have a policy for conducting regular competency assessment and training for malaria microscopists.*

1. INTRODUCTION

1.1. Background

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans of the Plasmodium [1,2]. In humans, malaria is caused by *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi* [3].Every minute a child on the African continent dies due to malaria [4].Approximately 68%the people live in malaria-endemic areas in Ethiopia, chiefly at altitudes below 2,000 meters above sea level[5]. The current population of Ethiopia is estimated at 96,633,458 as of July 1 2014 and the population density is 87 people per Km^2 [6]. Five main malaria eco-epidemiological strata are recognized in the country; 1) Stable, year-round transmission in the western lowlands and river basins areas of Gambella, 2) Seasonal transmission in lowland areas below 1,500 meters, 3) Epidemic-prone areas in highland fringes between 1,500 and 2,500 meters, 4) Arid areas where malaria is only found near semi-permanent water bodies 5) Malaria-free highland areas above 2,500 meters [5].

Correct diagnosis is vital for the malaria prevalence and incidence indicators used to evaluate the impact of malaria control interventions[7].Prompt and reliable laboratory diagnosis is recognized as an important component of effective malaria case management and control [8,9].Strengthening laboratory diagnosis should help to reduce malaria morbidity and mortality [10].

Malaria is typically diagnosed by the microscopic examination of blood using blood films [11,12]. Microscopy is the most commonly used method to detect the malaria parasite, about 165 million blood films were examined microscopically per year for malaria in 2010[13]. The detection of Plasmodium parasites by light microscopy is still the primary method of malaria diagnosis in most health care facilities throughout the world [14].

According to the Services Provision Assessment survey on the availability and functionality of health facilities carried out in EFY 2006(2013/2014), there are a total of more than 3471 health facilities in Ethiopia. Among those about 3,315 were Health centers and 156 Hospitals which are functional currently [15]. In Ethiopia, health services are provided through a four-tiered system comprising specialized referral hospitals, zonal hospitals, district hospitals and primary health care units(comprises Health center and health post), with average catchment populations of about 5 000 000, 1 000 000, 250 000 and 25 000 people, respectively. From those health facilities in the tier system specialized referral hospitals, zonal hospitals, district hospitals and Health centers provides integrated laboratory services. In addition to those health facilities with integrated laboratory

services, there are National Reference laboratory and Regional reference laboratories which provide laboratory services [16].

Quality control programmes are a prerequisite of any laboratories including Malaria Microscopy Laboratory. The World Health Organization (WHO) recommends the cross-checking of laboratories using different methods such as Blinded rechecking, onsite evaluation and panel testing [14].WHO also recognizes the need to address the quality issue by developing country-adapted guidelines and tools for implementing External Quality Assurance for malaria microscopy through a network of health facilities. The strengthening of malaria microscopy through a network of health facilities in a district implies better-trained staff, enhanced material support, and a system for assuring quality of malaria microscopy services [17].The National Laboratory Quality System (NLQS) Operational Plan was developed by Ethiopian Public Health Institute (EPHI) in December 2006 to establish a system for ensuring high quality laboratory services for diseases such as malaria, HIV, and TB. Health facilities at all level of the tier system that are involved in malaria case management must participate in EQA [18].

Competency assessment is one of the method by which we can verify that our employees are competent to perform laboratory testing and report accurate and timely results. The goal of competency assessment is to identify potential problems with employee performance and to address these issues before they affect patient care. Training and competency assessment in malaria microscopy using already trained personnel and validated slides are important for evaluating, improving and maintaining performance in malaria microscopy [19]. Competency assessment is also an opportunity to provide continuing education and performance feedback to employees and to document valuable objective information for performance evaluations [20].

1.2. Statement of the problem

Globally, an estimated 3.3 billion people in 97 countries and territories are at risk of being infected with malaria and developing disease , and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year). According to the latest estimates, 198 million cases of malaria occurred globally in 2013 (uncertainty range 124–283 million) and the disease led to 584,000 deaths. Of these 90% of all malaria deaths occur in sub-Saharan Africa [21]. Young children are mostly at risk for severe disease, particularly in high transmission zones, since their immunity has not yet been fully developed [22]. Globally, the disease caused an estimated 453 000 under-five deaths in 2013. In 2013, an estimated 437 000 African children died before their fifth birthday due to malaria [23]. Malaria is also the leading cause of morbidity and mortality in Ethiopia. It was the leading cause of outpatient visits,

health facility admissions and inpatient deaths, accounting for 12% of reported outpatient visits and nearly 10% of admissions [5].

Even though light microscopy, established over 100 years ago and frequently considered the reference standard for clinical diagnosis, it has been neglected in control programmes and evidence suggests field standards are commonly poor[24].Expert microscopist is not always consistent with respect to a number of aspects that can have a direct effect on the results obtained. An important example of this inconsistency is in the length of time a slide is reviewed [25].

Despite its widespread uses, diagnosis by microscopy suffers from two main drawbacks: many settings (especially rural) are not equipped to perform the test, and the accuracy of the results depends on both the skill of the person examining the blood film and the levels of the parasite in the blood. The sensitivity of blood film examination microscopically ranges from 75–90% in optimum conditions, to as low as 50%. [13].

The lack of qualified professionals to correctly diagnose malaria and the lack of quality control in the laboratory diagnostic process have been identified as the main reasons for the lack of success in the current strategy to control malaria [26].Quality Assurance (QA) programmes for malaria microscopy are not adequately developed. Competency evaluation of each microscopist has the potential to significantly improve the QA of malaria diagnosis, raise confidence and enhance career development but this is not observed in most countries [27].

Laboratory employees solve problems very often but are frequently not aware that they are doing an error. The main reasons for this are lack of conducting competency and lack of follow up regularly. One of the challenges for any laboratory in establishing a competency assessment program is defining the extent of assessment that will be performed in each area and lack of any established system on how to conduct competency assessment [28].

Although tremendous progress has been made by the Federal Ministry of Health (FMoH) in the laboratory diagnosis of malaria, critical gaps and challenges have emerged with rapid program expansion of intervention coverage and services. The major gaps include poor malaria laboratory diagnostic capacity and associated supplies, lack of quality assurance and a monitoring system. Many rechecking laboratories(regional referral laboratories, sub regional laboratories, selected hospital laboratories, and selected health center laboratories) were established in our country in different regions to recheck the blood film slides examined at microscopic sites, but there is no evidence that really those malaria microscopists working at rechecking sites are competent to recheck blood film

slides examined at testing sites. Therefore this study aimed to assess the Performance of Malaria Microscopists working at Malaria EQA Rechecking Laboratories.

1.3. Literature Review

A prospective study conducted in Pakistan to evaluate the performance of laboratories over seven months period in four districts showed that, from a total of 8,118 slides examined of which 209 (2.6%) were found positive for malaria parasites (slide positivity range between 1.6% to 6.0%). The District Laboratory Supervisors in four districts reexamined a total of 1,770 slides (22%). The proportion of slides found discordant ranged from 0.5% to 1%. The quality of smear preparation was found acceptable in 73% slides. According to this study, there is a low-discordance result on BF microscopy examination which is an expected, while the quality of smear preparation is lower than expected [10].

A cross sectional study conducted in USA on the WHO55 test, the device scored a “Level 4” using the WHO published grading scheme. Broken down by more traditional analysis parameters this result was translated to 89% and 70% sensitivity and specificity, respectively. Species were correctly identified in 61% of the slides and the quantification of parasites fell within acceptable range of the validated parasitaemia in 10% of the cases. A pooled analysis of the 174 slides used for both tests resulted in an overall 92% sensitivity and 90% specificity with 61% species and 19% quantifications correct [25].

A 4 years (1995-1998) survey conducted in Canada showed that the result of 1995 survey indicated shortcomings in detection and identification of malarial parasites in blood films. In addition a 1997 follow-up external quality assessment survey indicated that problems persist as 27% of laboratories failed to correctly speciate *P. falciparum* [29].

A Retrospective study of 7 surveys conducted in South Africa from January 2000 to August 2002 showed that the mean percentage incorrect result rate was 13.8% (95% CI 11.3-16.9%), which is alarmingly high, with about 1 in 7 blood films being incorrectly interpreted. Most participants with incorrect blood film interpretations had acceptable Giemsa staining quality, indicating that there was less of a problem with staining technique than with blood film interpretation [30].

A cross sectional study conducted in Democratic Republic of the Congo between August and September 2010 showed that from 174 participant laboratories; 59.2% of participants scored correct results and another 16.1% reported minor errors, all but one were errors in parasite density (reporting "+++" instead of the expected "++++" score. By contrast, for parasite-negative sample, 24 (16.7%) of participants reported the presence of Plasmodium parasites, mostly *P. falciparum* [14].

A cross sectional study was conducted in Zambia to assess whether microscopy improves the management of febrile persons in six health centers. Staff interviews and a blinded review of a series of blood slides from each facility by two expert microscopists were also conducted. Of 1,442 outpatients, 655 (45%) reported fevers or had a temperature > or = 37.5 degrees C. Blood slide microscopy was ordered in 28-93% of patients with fever (mean = 46%). Eighty-eight (35%) patients without parasitemia were prescribed an antimalarial drug. Antimalarial drugs were prescribed with equal frequency to those who were referred for a blood slide (56%) and those not referred (58%). The sensitivity of microscopy was 88% and the specificity was 91%[31].

A cross sectional study conducted in Hawassa Town, Ethiopia showed that from a total of 72 participants, 14 (19.4%) of the participants correctly reported all the ten distributed slides, whereas 58(80.6%) missed at least one slide. Overall, the sensitivity and specificity of participants in detection of malaria parasites were 82% and 96.5% respectively. The overall agreement between participants and reference readers on detection of malaria parasite was 88% (Kappa = 0.76) while on identification of malaria species was 74.3% (kappa = 0.63). Lower agreement on detection and identification of slides with low parasitic density and mixed infection were observed. Agreement was relatively lower for government health centers (69%; kappa = 0.56). None of the participants reported parasitic load per micro liter method [32].

The study conducted in five health centers and one hospital in north Gondar zone in Ethiopia showed that, out of 3625 patients whose blood film was sent, the proportion of *P. falciparum* and *P. vivax* was 64.6% and 35.6%. The specificity and positive predictive values were 73.7% and 58.1 %, respectively. Agreement between the operational readers and the reference was 75%. The chance of corrected agreement or kappa score was 0.47 [33].

A cross sectional study conducted in public health facilities in North Gondar from March 2013 to April 2013 showed that from a total number of 297 subjects, 61.6% (183/297) patients tested positive for malaria by Giemsa microscopy of which, 72.1% (132/183) and 27.9% (51/183) were diagnosed as *P. falciparum* and *P.vivax*, respectively. By nPCR, 73.1% (217/297) were malaria-positive. Among microscopy-negative samples, 13.1% (39/297) samples turned malaria-positive in nPCR. In nPCR, the rate of mixed Plasmodium infections was 4.7% (14/297) and 3.03% (9/297) were positive for *P.ovale*. Using nPCR as reference the sensitivity, specificity, positive predictive and negative predictive values of Giemsa microscopy were 82.0%, 93.8%, 97.3% and 65.8%, respectively, with a good agreement (κ=0.668) to nested PCR. The sensitivity and specificity of Giemsa microscopy in identifying *P. falciparium* infections were 74.0% and 87.4% and 63.2% and 96.5% for *P. vivax* infections, respectively [34].

1.4. Significance of the study

- Used to provide information about the performance of Malaria Microscopists who are working at Malaria Rechecking Laboratories in the country.
- Provide information which is used to improve the performance of Malaria Rechecking Laboratories and Malaria microscopic center based on identified gaps.
- Provide information which is used to prioritize gaps identified and to provide training based on the gaps identified.
- It provides information for policy developers, Universities, Medical colleges, National and Regional organizations, FMoH, and authorized bodies to work hard from their side based on the study findings.

2. OBJECTIVE OF THE STUDY

2.1. General Objective

To Assess the Performance of Malaria Microscopists and Malaria EQA Rechecking Laboratories in Ethiopia.

2.2. Specific Objectives

- To assess parasite detection, species identification and parasite quantification capabilities of malaria microscopists among different socio demographic characteristics.
- To assess blood films staining quality of the laboratories.
- To assess the performance of laboratories using blinded rechecking.

3. MATERIALS AND METHODS

3.1. Study Design

A cross-sectional study was conducted to assess the performance of Malaria Microscopists working at Malaria EQA Rechecking Laboratories in Ethiopia from February 1 to May 10, 2015.

3.2. Study Area

This study was conducted in Ethiopia at malaria rechecking laboratories. Ethiopia has nine regions and two city administrators. The current population of Ethiopia is estimated at 96,633,458 as of July 1 2014 and the population density is 87 people per Km^2[6].Five main malaria eco-epidemiological strata are recognized in the country;1)Stable, year-round transmission in the western lowlands and river basins areas of Gambella, 2) Seasonal transmission in lowland areas below 1,500 meters, 3) Epidemic-prone areas in highland fringes between 1,500 and 2,500 meters, 4) Arid areas where malaria is only found near semi-permanent water bodies 5) Malaria-free highland areas above 2,500 meters[8]. The study was conducted at 23 malaria rechecking sites (Fig. 3. 1); 12 Regional Laboratories, 4 sub-Regional Laboratories (SRL),4 Hospital Laboratories and 3 Health Center Laboratories(HCL).

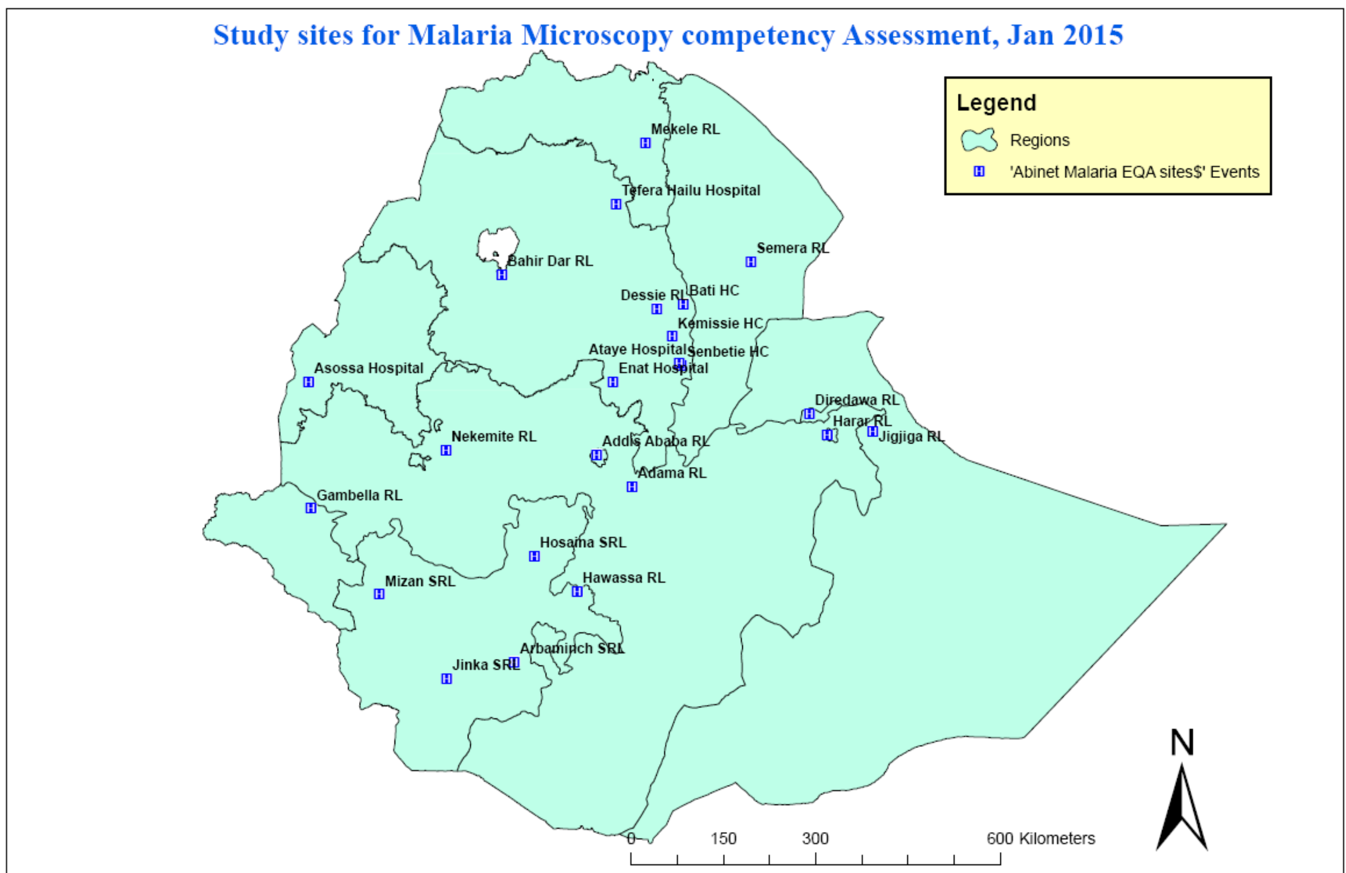

Figure 3.1: Schematic representation of malaria rechecking sites (study sites) in Ethiopia.

3.3. Study Period

The study was conducted from February 1to May 10, 2015.

3.4. Population

3.4.1. Source population

The source populations were all laboratory professionals who are working at malaria EQA rechecking laboratories in Ethiopia.

3.4.2. Study population

The study populations were all malaria microscopists who are working on malaria microscopy at malaria EQA rechecking laboratories in Ethiopia and those who were involved in this study.

3.4.2.1. Inclusion criteria

- Health facilities which were started rechecking blood film slides.
- All malaria Microscopists who are working at malaria EQA Rechecking Laboratories and those who were present during data collection were included in the study.

3.4.2.2.Exclusion criteria

- Microscopists who were working at EQA Rechecking Laboratories but not participating on Rechecking Malaria blood film slides and those who were not willing to give informed consent would be excluded from the study.

3.5. Sample Size

All Malaria Microscopists who were working at malaria rechecking laboratories and those who were available during the study time were included in the study. There were a total of 107 Malaria Microscopists who were participated on the Study.

3.6. Study Variables

3.6.1. Dependent Variables

- Ability to detect plasmodium parasite
- Ability to identify plasmodium species
- Ability to identify Stage of plasmodium parasite
- Ability to Quantify plasmodium parasite

3.6.2. Independent Variable

- Work experiences
- Educational status(Level)
- Training (Trained or not). This training may be Training of Trainers (TOT) or Basic training on Malaria Laboratory Diagnosis and Quality Assurance which is provided nationally or at Regional Level.
- Types of health facility (Regional Reference Laboratory (RRL), Hospital Laboratory (HL), and Health Center Laboratory (HCL)) with Performance.
- Type of Services(Provide Routine Malaria Microscopy service)

3.7. Measurement and Data Collection

3.7.1. Panel Slide Preparation and Distribution

3.7.1.1. Whole Blood Collection, Smear Preparation and Staining

3-5 ml of whole blood was collected from volunteers and those who were above 18 years into EDTA tubes. Blood donors for BF smear preparation were either known to have no risk for malaria (confirmed negative for Plasmodium species parasite), and from those screened and found to have blood smears positive for malaria parasites. Sample from Negative donors was collected at EPHI while sample from positive donors was collected at Adama malaria center. Venipuncture blood was collected from volunteer donors by trained technicians using blood drawing SOPs and by applying Universal safety Precautions.

Blood smears were made from anti-coagulated blood within 6 hours after collection. A total of 200 blood film slides and one Dried Blood Spot (DBS) were prepared from each donor. Both thick and thin blood films were prepared on a single slide. Six microlitre or one large drop and two microlitre or one small drop was used to prepare thick and thin blood film, respectively. After making the requisite number of slides and preserving blood blots on filter paper, the remaining blood was discarded in biohazard containers safely. The thin blood film was fixed with absolute methanol after well dried and then both thin and thick were stained with 3% Giemsa working solution for 30-45minutes.Finally Blood film for microscopic diagnosis of malaria was made semi-permanent by cover slipping using appropriate mounting medium and cover glass.

3.7.1.2. Blood Film Slide Validation and Characteristics

Six Malaria Microscopy experts were involved on the validation and each of them was read 2 blood film slides from each donor. This means that 12 blood film slides per each donor were used for validation. The validaters were Malaria microscopists at national level and from Hydas World Health [HWH]). EPHI had an agreement with Hydas World Health [HWH]) to validate Blood Film Slides which were used for slide bank. This work was the part of slide bank establishment so blood film slides were sent to HWH based on this agreement (Annexed). The validators were interpreted the blood film smears using four diagnostic criteria: 1) The presence or absence of malaria parasites; 2) Identification of the species of parasites; 3) Identification of stage; 4) Quantification of parasitic load. Quantification was performed on thick blood film against 200 white blood cells

$$\text{Parasites}/\mu\text{L} = \frac{\text{Parasites counted}}{\text{WBCs counted}} \times \text{WBCs}/\mu\text{L}$$

[8].

In addition PCR was used to handle any discordant results among validators and for further confirmation of results. Those activities were performed as a part of Malaria Slide Bank which was established at a National level. Blood film slides for Negative, *P. Falciparum*, *P. Vivax* and Mixed (*P. Falciparum* and *P. Vivax*) were validated with in this process. While BF Slides for *P. Malerea* and *P. Ovale* were obtained from international PT providers such as Digital PT from Canada and WHO National Institute for Communicable Disease (WHO/NICD) Malaria PT from South Africa.

3.7.1.3. Administration of Blood Film Slides

After Validation, the following set of blood film slides with different values was given to study population. Based on WHO recommendation, two types of composition were used: 1) Composition used for assessment of presence/absence of parasites, species, and stage identification, and 2) Composition used for assessment of quantification.

Slide set 1 (10 slides): Assessment of parasites detection, stage and species identification

- 2- negative slides
- 8- positive slides of low densities
    - 3 *Plasmodium falciparum* slides with different density
    - 2BF slide with *Plasmodium v*ivax slides
    - 1 Mixed species slide (Include *P. falciparum* and *P. Vivax*. co infecting species according to local prevalence)
    - 1 BF slide with *Plasmodium malariae* (obtained from international PT providers), and
    - 1 BF slide with *Plasmodium ovale* slide (obtained from international PT providers).

Slide set 2 (2 positive slides): Used for the Assessment of quantification

- 1 *P. falciparum* with a parasite density of 1891 P/µL (with the range of 1419-2363 parasites/µL)
- 1*P. falciparum* with a parasite density of 50,659 P/µL (with the range of 37990-63323 parasites/µL)

Based on WHO recommendation, quantification results of participants which were between 25% $\pm$ the mean calculated from result of expert readers was considered as correct quantification result. A total of 120 minutes (10 minutes per BF slides) were allocated for those 12 BF slides [35]. Based on the number of staffs and workload, a total of two days were given for each rechecking sites to examine those BF slides and then the data collector retrieved the slides after each participant completed the tests.

3.7.2. Blinded Rechecking and Onsite Evaluation

Both blinded rechecking and on site evaluation are EQA methods used to assess the performance of laboratory. Blinded rechecking was done at those laboratories which were used as Malaria EQA rechecking laboratories and perform BF microscope examination routinely. It was performed by collecting a total of ten BF slides (5 positive and 5 negative BF slides) randomly from laboratory log book. From a total of 23 study sites 7 of them (4 Hospital and 3 Health center laboratories) which were providing routine service on malaria microscopy were assessed based on this method. Blood film slides were randomly selected from the log book by one of data collector and then provided to the other data collector member to read it blindly. Any discordant result was confirmed by the second data collector, and then data collectors were discussed and agreed on discordant result with the laboratory professionals working in that specific laboratory. In addition to analysis of concordant and discordant result, good and poor BF slides were evaluated based on the criteria (Annexed).

Onsite supervision includes assessment of laboratory supplies storage and inventory, basic procedures, quality of reagents, training status of the laboratory staff, performance of internal QC and result recordkeeping practice. This was conducted using a standardized check list (Annexed). The whole laboratory process; specially staining quality was assessed using two unstained thin blood film slides with the following composition.

- 1 *P. Falciparum* BF slide with an average Parasite density of (24,000p/micro liter)
- 1 Negative BF slide

Then stained BF slides was evaluated by Principal investigator or a person who was collecting the data against the criteria of good quality blood film staining set by WHO(Annexed).

3.7.3. Questionnaire

A structured and standardized questionnaire which was used to address information about participating facilities and Malaria Microscopists was distributed to study population. The questionnaire had sub-components like: age, sex, educational background, type of health facility, in service training, work experience, routine practice of the professionals, and number of slide rechecked per person. The data was collected by trained and those who had good experience on Malaria Microscopy. The questionnaire was pre-tested before the main study. No personal and health facility identifier was included. Individuals were given a unique code numbers.

3.8. Data Management and Quality Assurance

The questionnaires were prepared in clear and understandable way, training was given for data collectors, and pre- testing of each questionnaire type was done at 5%of the study population. The clarity, understandability and flow of each question were assessed properly. Intensive supervision was done during data collection. The quality of the blood film slides was properly checked against WHO standard before provided to malaria microscopists for reading and the results was critically seen for completeness.

3.9. Statistical Analysis

Data was collected and entered into Microsoft Excel sheets and exported to software SPSS version 20 for analysis. Level of performance in detections, species identification and quantification of malaria parasite was compared with independent variables. Association was taken as significant at $P < 0.05$.False positive rate (% false positives) and false negative rate (% false negatives) were calculated to see the discordant results. Chi-square (for categorical data), sensitivity, specificity, percent agreement, and kappa score were calculated to assess laboratory professionals' performance in detecting and identification of Plasmodium species using light microscopy. Based on WHO recommendation, microscopists were classified as: "In training"- when the agreement with the expert reader in detection with species identification of malaria parasite was less than 70%; "Advanced"- when the agreement was greater than or equal to 70% but less than 80; "Reference"- when the agreement was greater than or equal to 80% but less 90%; and "Expert" -when the agreement was greater than or equal to 90% [35]. Kappa Value was calculated (Kappa, $K=\frac{(po-pe)}{(1-pe)}$) to see the strength of an agreement.

Based on the calculation the strength was classified as: < 0.20 Slight agreement, 0.21– 0.40 Fair agreement, 0.41–0.60 Moderate agreement, 0.61–0.80 Substantial agreement, 0.81–0.99 Almost perfect agreement [36]. A Percent agreement score ≥80% is a passing point for external quality assessment based on the National Guidelines [18]. All participants who were scored an agreement point less than 80% will get the training by EPHI after submitting the final thesis.

3.10. Ethical Clearance

This study was done after getting ethical clearance from the ethical clearance committee of Addis Ababa University College of health Sciences, Department of Medical Laboratory Sciences, departmental research and ethics review committee. Information about the study was provided to all Malaria Microscopists involved in the study and to all concerned bodies found at all studies area. All participants were assured about the confidentiality, protection and anonymity of the data. Written informed consent was obtained from voluntary study participants before conducting the study.

3.11. Dissemination of Results

This thesis was submitted to College of Health Sciences, Department of Laboratory Sciences. So it can serve as a reference in the library. In addition, a copy of this material was given to EPHI, FMoH, and Regional Health Bureaus. So that it may help concerned bodies to develop or revise policies based on the study findings. It will be presented to all study participants and summary result will be provided for each health facilities as a feedback. This result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in related workshops and seminars.

3.12. Staffing and Budget Required

This study was done by Principal Investigator in collaboration with other data collectors; those who had training on malaria microscopy and who had more than five years' experience on malaria microscopy. The cost of the study, which was about 230,000.00 Ethiopian birr was covered by EPHI, and Addis Ababa University.

4. RESULTS

From a total of 129 Malaria microscopists who were participating on rechecking of blood film slides, 107(83%) were available during the study time and all of them were participated in the study. Based on the data, the mean age of the participants was 30 (SD = 5.04) years and about 90 (84.1%) participants were males (Table 4.1).

Table 4.1: Demographic characteristic of malaria microscopists working on malaria microscopy at malaria EQA rechecking laboratories in Ethiopia (N=107)

Variables	Number	Percent	Cumulative Percent
Age in Year			
20-30	68	63.6	63.6
31-40	37	34.6	98.2
≥41	2	1.9	100.0
Sex			
Male	90	84.1	84.1
Female	17	15.9	100.0
Educational Status			
Diploma	25	23.4	23.4
Degree	63	58.9	82.3
MSc and Above	19	17.8	100.0
Work Experience on Malaria Microscopy			
<2 Years	4	3.7	3.7
2-5 Years	43	40.2	43.9
> 5 Years	60	56.1	100.0
Place of Work			
Health Center Laboratory	16	15.0	15.0
Hospital Laboratory	22	20.5	35.5
Sub Regional Laboratory	15	14.0	49.5
Regional Reference Laboratory	54	50.5	100.0
Participation on Malaria Microscopy and QA Training			
Yes	83	77.6	77.6
No	24	22.4	100.0
Number of BF Slides Rechecked per Person/ Year			
≤150	56	52.3	52.3
151-300	39	36.4	88.8
301-450	6	5.6	94.4
≥451	6	5.6	100.0

Based on WHO recommendation 78(72.9) participants were in training on species identification. There was no any participant with expert classification based on the recommendation. Eleven (10.3%) participants were quantified both BF slides correctly while 17(15.9) participants were missed both BF slides and 34(31.8%) participants were used unrecommended quantification system (Table 4. 2).

Table 4.2: Overall Performance of malaria microscopists on detection, species identification, stage identification and parasite quantification with their classification based on WHO recommendation (N=107)

Performance Classification	Frequency	Percent	Cumulative Percent
Percent Agreement on Species Identification			
≥90%(Expert)	0	0	0
≥80% but less than 90%(Reference)	11	10.3	10.3
≥70% but less than 80%(Advanced)	18	16.8	27.1
<70%(In Training)	78	72.9	100
Percent Agreement on Stage Identification			
≥90%	33	30.8	30.8
≥ 80% but less than 90%	35	32.7	63.6
≥70% but less than 80%	27	25.2	88.8
<70%	12	11.2	100.0
Performance on Parasite Quantification			
Quantified both BF slides correctly	11	10.3	10.3
Quantified only one BF slide correctly	45	42.0	52.3
Missed both	17	15.9	68.2
Used unreccomended quantification system	34	31.8	100.0

From a total of 1070 blood film slides 1036(96.8%) were correctly reported for the presence and absence of malaria parasite and from a total of 856 positive blood film slides only 486(56.8%) of them were reported correctly on species identification by participants. Of 214 BF slides which were distributed for quantification, only 63(29.4%) of them were quantified correctly (Fig.4. 1).

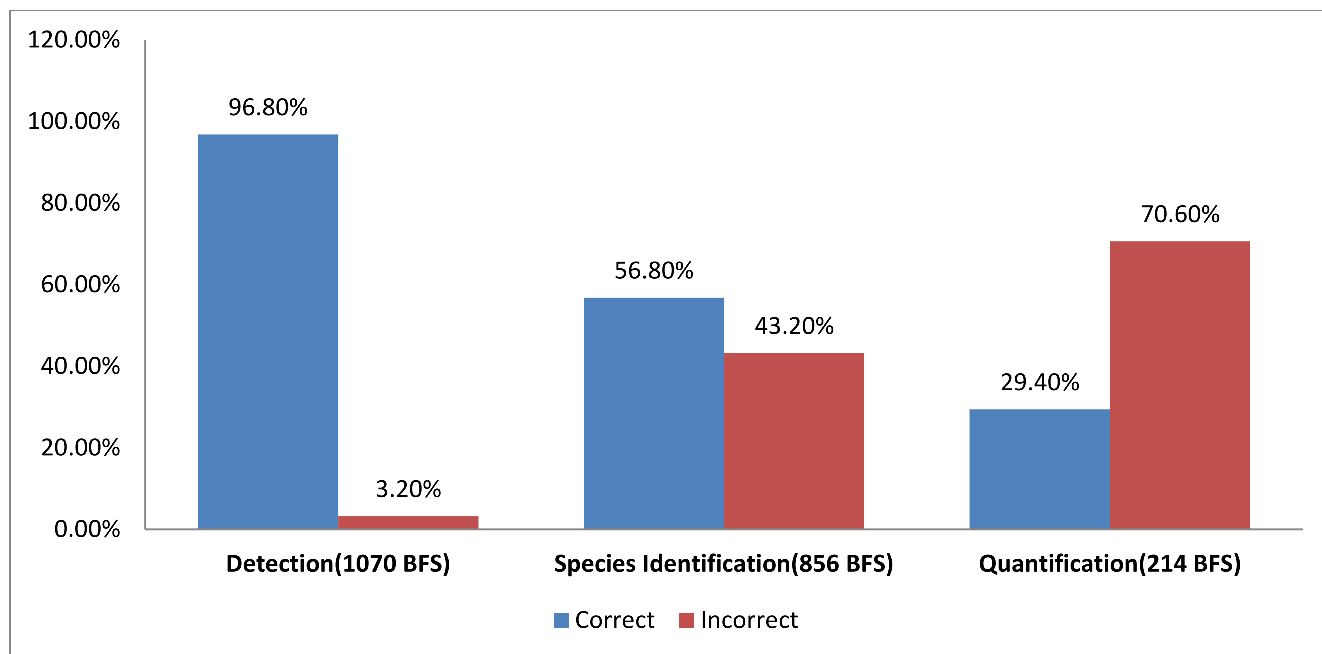

Figure 4.1: Over all summary of participants' performance on detection, species identification and quantification

Overall, the sensitivity and specificity of participants in detection of malaria parasites were 96.8% and 96.7%, respectively. The overall agreement on detection of malaria parasite was 96.8% (Kappa = 0.9) which is almost 'perfect agreement' (Table 4. 3). False positive rate (Negative BF slides reported as positive) and false negative rate (positive slides reported as negative) were 0.84% and 11.5%, respectively.

Table 4.3: Over all sensitivity, specificity and agreement of participants in detecting of malaria parasite based on the total number of observations

Participant Reader		Expert Reader			Sensitivity	Specificity	Agreement	Kappa
		Positive	**Negative**	**Total**				
Parasite Detection	Positive	829	7	836	96.8%	96.7%	96.8%	0.9
	Negative	27	207	234				
	Total	856	214	1070				

Overall, the sensitivity and specificity of participants in species identification of malaria parasites were 56.7% and 96.7%, respectively. The overall agreement on identification of malaria species was 64.77% (kappa = 0.33) which is a 'fair agreement' (Table 4. 4).

Table 4.4: Over all sensitivity, specificity and agreement of participants in species identification of malaria parasite based on the total number of observations

Participant Reader		Expert Reader			Sensitivity	Specificity	Agreement	Kappa
		Expected Correct species	**Negative**	**Total**				
Species Identification	Reported species	486	7	493	56.7%	96.7%	64.77%	0.33
	Negative	370	207	577				
	Total	856	214	1070				

The performance of participants on species identification showed that 80.07% of BF slides with *P. falciparum*, 64.95% of BF slides with *P. vivax*, 32.7%of BF slides with *P.ovale*, and 2.8% of BF slides with *P. malerea* were identified correctly (Fig. 4. 2). *P. malerea* and *P.ovale* were the least malaria species which were identified correctly. A total of 64(61.5%) BF slides with *P malerea* were identified wrongly as *P.falciparum*, and 42(58.3%) BF slides with *P.ovale* were identified wrongly as *P.vivax*.

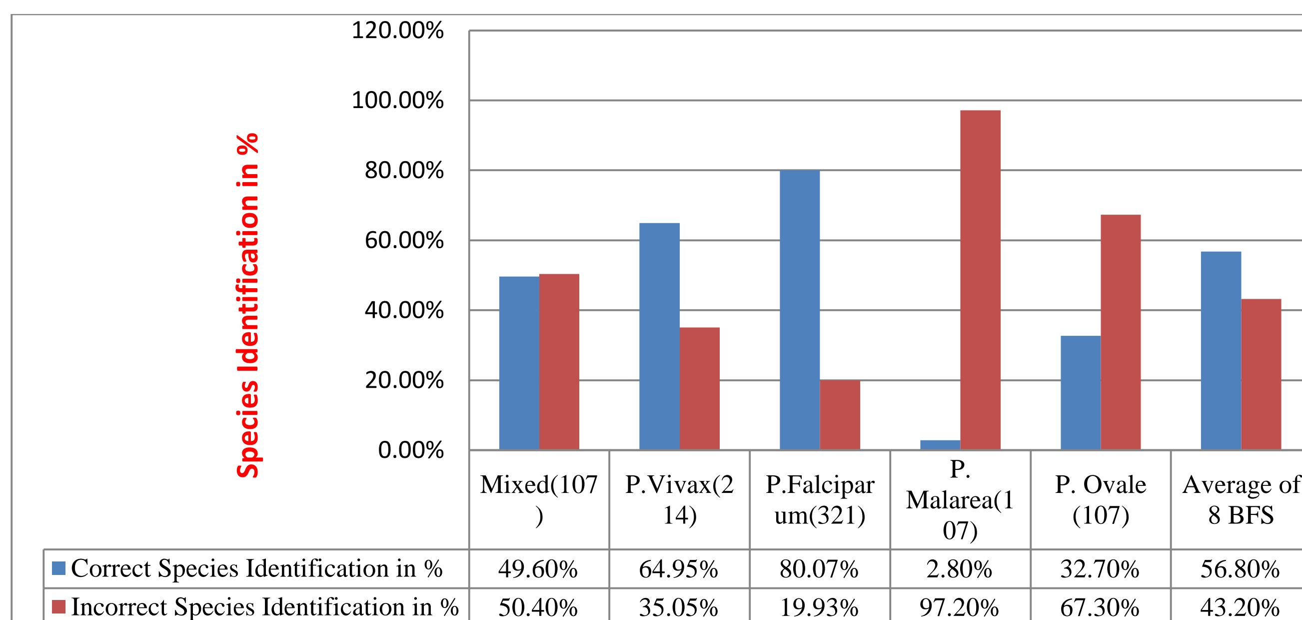

Figure 4.2: Performance of malaria microscopists on each species identification (8 different BF slides with 4 malaria species/person)

From a total of 107 participants 97(90.7%) were scored <80% which was failed based on WHO and National Guideline [20,29]. The relationship between participants performance with educational statutes showed that, the number of failed participants (<80%) was higher among participants with diploma 25(100%) followed by participants with degree 59(93.6%) and participants with MSc and

above 13(68.4%). The difference in educational status to score above or below the passing score (80%) was significant (P=0.001). The number of participants who scored ≥80% higher among participants with >5years work experience9 (15%) followed by 2-5years 1(2.3%) and<2 years work experience, respectively (P=0.75). The number of participants who scored ≥80% was higher among participants who had malaria microscopy and QA training 10(12.1%) (Table 4.5).

Table 4.5: Relationship between some demographic characteristic with Passed (≥80%) and Failed (<80%) score on species identification based on WHO and National Guideline (N=107)

Variables	Greater or Equal to 80%(Passed)	Less than 80%(Failed)	Chi-Square	Degree of freedom	p-value
Educational Status					
Diploma	0	25	14.330	2	.001
Degree	4	59			
MSc and Above	6	13			
Work Experience					
<2 Years	0	4	5.178	2	.075
2-5 Years	1	42			
> 5 Years	9	51			
Place of Work					
Health Center Laboratory	0	16	4.508	3	.212
Hospital Laboratory	3	19			
Sub Regional Laboratory	0	15			
Regional Reference Laboratory	7	47			
Participation on Malaria Microscopy and QA Training					
Yes	10	73	3.190	1	.074
No	0	24			

The sensitivity of participants at regional reference laboratory on the identification of malaria species was 53.2% which was lower than the sensitivity of participants from other rechecking sites. Participants who were working at hospital laboratory had higher percent agreement which was 72.3 %(Kappa=0.51) while regional reference laboratory had the least (61.7%, Kappa=0.46) agreement (Table 4.6).

Table 4.6: Agreement of participants with expert reader in identification of malaria species by type of health facility (N=107)

Variable		Participant Reader	Expert Reader			Sensitivity	Specificity	Agreement	Kappa
			Positive	Negative	Total				
Type of Health Facility	Regional Reference Laboratory	Positive	230	5	235	53.2%	95.4%	61.7%	0.46
		Negative	202	103	305				
		Total	432	108	540				
	Sub Regional Laboratory	Positive	66	0	66	55%	100%	64%	0.47
		Negative	54	30	84				
		Total	120	30	150				
	Hospital Laboratory	Positive	115	0	115	65.3%	100%	72.3%	0.51
		Negative	61	44	105				
		Total	176	44	220				
	Health Center Laboratory	Positive	75	2	77	58.6%	93.8%	65.6%	0.49
		Negative	53	30	83				
		Total	128	32	160				

Quantification performance of malaria microscopists working at sub regional laboratory was 73.3% which was higher than malaria microscopists performances from others malaria rechecking laboratories significantly (P=0.003).Study participants who were participated on malaria microscopy and QA training had a better performance on quantification than those who had no training. The difference was significant (P=0.000). Quantification capability of malaria microscopists with MSc was 57.9% which was higher than degree (53.9%) and diploma (44%)(Table4.7).

Table 4.7: Relationship between quantification performance of malaria microscopists with work experience, educational status, types of health facility and training (N=107).

Variables	Quantified at least one BF slide	Missed both BF slides	Chi-Square	Degree of freedom	p-value
Educational Status					
Diploma	11	14	.999	2	.607
Degree	34	29			
MSc and Above	11	8			
Work Experience					
<2 Years	2	2	.390	2	.823
2-5 Years	21	22			
> 5 Years	33	27			
Place of Work					
Health Center Laboratory	6	10	14.239	3	.003
Hospital Laboratory	5	17			
Sub Regional Laboratory	11	4			
Regional Reference Laboratory	34	20			
Participation on Malaria Microscopy and QA Training					
Yes	54	29	24.016	1	.000
No	2	22			

From a total of 23 malaria rechecking laboratories only 7(30.4%) laboratories were providing routine service on malaria microscopy and which were stored BF slides for rechecking. Ten (5 negative and 5 positive) BF slides were randomly selected and rechecked blindly from each laboratories. Out of 70 BF slides which were rechecked at seven laboratories, there was only 1(1.4%) discordant result on species identification. Based on a criteria set for good and poor BF slides, 44(62.8%) of BF slides were poor blood film slides.

Concerning laboratory supplies used for diagnosis of malaria parasite using a microscope; PH meter and PH tablet were not available at 22 (95.6%) rechecking laboratories, while distiller (distilled water) and brown bottle were not available at 14(60.8%) rechecking laboratories. Malaria rechecking laboratories had an average of 7(with the range 2-15) functional microscopes. From a total of 23 malaria rechecking laboratories 4(17.4%) of them had no any Giemsa reagent. Out of 19 laboratories which had reagent, all of them were performed quality control when they were opening the new batch (container). Based on the evaluation criteria of good BF slide (Annexed), Giemsa reagent at 9(47.4%) laboratories had poor blood film staining quality.

5. DISCUSSION

Malaria is still a leading cause of morbidity in Ethiopia where 68% of its population is at risk. Microscopy of Giemsa stained thick and thin blood films is the standard for the diagnosis of malaria. Competency assessment is one of the method by which we can verify that our employees are competent to perform laboratory testing and report accurate and timely results. In the current study which was conducted to assess the performance of malaria microscopists, an agreement between participants and malaria microscopy expert readers in the detection of malaria parasites was 96.8% which was relatively higher when compared with the study conducted in Hawassa town, Ethiopia which was 88% [32]. An overall agreement kappa value of our study finding on detection of malaria parasites with expert readers was 0.9 which is classified as 'almost perfect agreement' based on kappa index[36], and it was higher than the study conducted in Hawassa town, Ethiopia (Kappa=0.67) which is defined as 'substantial' [32]. Based on WHO recommendation 78(72.9%) participants were 'in training' in the current study while only 17(23.6%) participants were rated as 'in training" in the study conducted in Hawassa town [32]. There was no any participant with expert level performance in our study while the study conducted in Hawassa town there were 18(25%) participants who were classified as expert [32]. This may be due to lack of providing routine service; which is used to develop malaria microscopists skill on malaria parasite detection and identification, or lack of regular training on malaria microscopy.

Overall, the sensitivity and specificity of laboratory professionals in detecting malaria parasites were 96.8% and 96.7%, respectively. Both the sensitivity and specificity of this study were higher than the study conducted in Hawassa town, Ethiopia which was 82% and 96.5[32], the study conducted in USA which was 92% and 90%[25], and the study conducted in Zambia which was 88% and 91% [31] for sensitivity and specificity, respectively. The higher sensitivity in this study on the detection of parasites indicates that there were low false negative results which mean that there were low misdiagnoses of true infections. The specificity in those three studies were very close to each other and they were above 96% which means that there were very low false positive results or the ability of participants to identify uninfected individual with malaria parasite were very high. This can help an individual to take an appropriate treatment which is complement with their infection which means that it reduce unnecessary treatment with anti-malarial drug for those who were not infected with malaria parasite.

Overall, agreement in identification of different species of malaria in the current study was64.77% which was lower than the study conducted in Hawassa town with percent agreement of 74.3% [32]. The strength of agreement in our study (kappa = 0.33) was lower than the finding reported in North

Gondar (kappa = 0.47)[33] and Hawassa town (Kappa=0.63)[32].From a total of 856 positive blood film slides only 486(56.8%) of them were reported correctly on species identification by participants. Species identification reported correctly in our study was lower than the study conducted in USA which was 61%[25]. From a total of 321 BF slides with *P. falciparum* 20%of them were failed to be identified correctly. The number of participants failed to correctly speciate *P. falciparum* in the current finding was lower than the follow-up external quality assessment survey conducted in Canada 27% [29]. The least malaria species which were identified correctly by the participants on this study was *P. Malerea* which was 2.8%. Blood film slides with *P.ovale* which were identified correctly (32.7%) was higher than the study conducted in North Gondar, north-west Ethiopia in which no one was correctly identified *P.ovale*[34]. This may be due to the fact that some participants did not spend sufficient time to examine the slides, or they were not using different references to differentiate the species, or they had no access to update themselves, or possibly they were not expect that *P. malerea* and *P. ovale* malaria species were not found in our country.

Our findings showed that 34.6% of cases with mixed (*P. falciparum* and *P. vivax*) BF slides were reported incorrectly as *P. falciparum*. Similar to our finding the study conducted in Hawwasa town showed that most mixed cases were reported as *P. falciparum* [32]. This may be due to the fact that some participants did not spend sufficient time to examine the slides, or possibly due to lack of regular training. Lack of correctly identifying the species may lead to incorrect administration of first line treatment. For example the recommended first-line treatment of all clinically and parasitologically diagnosed uncomplicated *P. falciparum* malaria in Ethiopia is an Artemisinin-based Combination Therapy (ACT) called Artemether-Lumefantrine (AL) while the first line treatment for *P. vivax* is oral chloroquine [5] . Correct species identification can be used to treat an individual with an appropriate first line drug and used to prevent drug resistance.

In our finding false positive rate (Negative BF slides reported as positive) was 0.84% which was lower than the study conducted in Canada (2.0%) [29], 6.9% reported in Hawassa town [32], 7% reported in USA [25], and 24.6% reported in Democratic Republic of Congo [14]. These false positive results could lead to unnecessary treatment with anti-malarial drug or a delayed diagnosis of the true cause of illness and confuse the clinician from considering other causes of fever and disease. False negative rate (positive slides reported as negative) in our study was 11.5% which was lower than 16.3% reported in Democratic Republic of Congo [14] but higher than 3% reported in USA [25]. False negative results can lead to delayed treatment, development of serious complications and death or exposure to unnecessary treatment with other (not anti—Malaria) drugs.

In our finding 11(10.3%) participants were quantified both BF slides correctly while 17(15.9%) participants were missed both BF slides and 34(31.8%) participants were used unrecommended quantification system which was + system. Correctly quantified blood film slides in our study were 31.3% which was lower than 81% of correct quantification reported in USA[25]. Participants who were used unrecomonded quantification (+) system in this study was 31.8%, which was lower than the study conducted in Democratic Republic of Congo in which all of the participants used this quantification system [14]. Quantification performance of malaria microscopists working at sub regional laboratory which was 73.3% was higher than malaria microscopists performances from the other malaria rechecking laboratories significantly (P=0.003). Study participants who were participated on malaria microscopy and QA training had a better performance on quantification of at least one blood film slide (65%) than those who had no training (8%). The difference was significant (P=0.000). Similarly based on the study conducted in USA, most participants who were quantified correctly had malaria microscopy training [25]. Lack of parasite quantification using recommended system may be due to lack of updated information (lack of training), or lack of awareness on the advantage of quantitative quantification system over semi quantitative (+) system, or may be lack of commitment to give time for counting parasite. Measuring parasite density (quantification) can be used to monitor patient response to treatment and to study drug efficacy.

In the current study from a total of 70 BF slides which was rechecked at 7 laboratories, there was only 1.4% discordant results which was slightly higher compared with a prospective study conducted in Pakistan with proportion of slides found discordant ranged from 0.5% to 1% [10] but lower than discordant result reported in South Africa 13.8%[30]. According to our study, from a total of 70 BF slides which were collected from rechecking sites for blind rechecking, the quality of smear and stain of blood film was found acceptable only in37.2% of BF slides, which was very low compared with the study conducted in Pakistan73% [10]. Based on this study 62.8% of blood film slides prepared at study sites was poor, this might be because of different reason such as: skill of professional, quality of supplies and reagents, or lack of using standard operating procedures. Poor BF slide may increase false negative and false positive rate of the results.

Based on the data collected using on site supervision, from laboratory supplies which were used for the diagnosis of malaria parasite using a microscope; PH meter and PH tablet were not available at 22 (95.6%) rechecking laboratories, while distiller (distilled water) and brown bottle were not available at 14(60.8%) rechecking laboratories. This may be due to lack of awareness among malaria microscopists that those supplies had very significant value for the quality of malaria microscopy service, or due to poor purchasing system in the facility, or no allocated budget for laboratory supplies. Rechecking laboratories had an average of 7(with the range 2-15) functional microscopes.

From a total of 23 malaria rechecking laboratories 4(17.4%) of them had no any Giemsa reagent. Of 19 laboratories which had reagent, all of them were performed quality control when opening a new batch (container) but they were not performing in a weekly (regular) basis. This may be due to lack of providing routine services, or lack of consciousness on the importance of performing quality control regularly. Based on the evaluation criteria of good BF slide, Giemsa reagent at 9(47.4%) laboratories had poor blood film staining quality. This may be due to inappropriate storage condition, or possibly incorrect specification of reagent ordered for purchasing, or probably used inappropriate reagent containers, or may be due to lack of adhering SOPs for reagent preparation and handling.

6. LIMITATION OF THE STUDY

- This study was not able to include all malaria microscopists who were working at malaria rechecking site because they were not available during the time of data collection.
- The sample was not representative because some big regions had lower number of malaria rechecking sites when compared with some small regions.
- All malaria rechecking laboratories were not addressed by blind rechecking method because regional reference laboratories and sub-regional laboratories were not providing routine services and had no stored blood film slides for rechecking.

7. CONCLUSION AND RECOMMENDATION

Even though the performance of participants were good on detection of malaria microscopy, their agreement with expert microscopist in the identification of different malaria species and quantification were very low. Most participants were not identified *P. malerea* and *P.ovale* correctly. Agreement of diploma participants was very low on species identification. The ability to identify true positive was less among participants who were working at regional reference laboratory. Participants who were not trained on malaria microscopy had very poor performance on quantification. Quantification performance of microscopists working at health center laboratory and Hospital laboratory were very low. Most BF slides prepared at malaria rechecking hospital and health center laboratories were poor. Malaria rechecking laboratories had no some important laboratory supplies which were used to provide quality service on malaria microscopy. A Giemsa reagent found in half of malaria rechecking laboratories was not stain good blood film. Generally there were gaps at rechecking laboratories and among malaria microscopists working at those laboratories. Therefore, to fill those gaps all stakeholders at all levels have to work on the implementation of regular competency assessment and training policy. Demonstration BF slides used for malaria microscopy training have to comprise *P. malerea* and *P.ovale*. Regions which are using or which are going to use health center and hospital laboratories as rechecking sites have to evaluate the performance of laboratories using all external quality assessment methods and have to conduct competency assessment for malaria microscopists who are working at those rechecking sites. Regional reference laboratories and sub-regional reference laboratories should practice on positive blood film slides regularly to improve their skill on species identification. All laboratories should have all necessary supplies and reagents with good quality to provide accurate, reliable and timely result.

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ANNEXES

Consent Form (English version) for Blood Donors

My name is Abnet Abebe and I am MSc student in Medical Laboratory Science at Addis Ababa University. I am doing a research entitled “Performance Evaluation of Malaria Microscopists working at Malaria EQA Rechecking Laboratories in Ethiopia”**.**

The objective of the study is to assess the Performance of malaria microscopists working at EQA Rechecking Laboratories. 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 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 medical service. My signature below indicates that I agree to participate in this study.

Signature of Person Obtaining Consent   date of signature

____

Subject’s signature   date of signature

____

Consent Form for Malaria Microscopists

My name is Abnet Abebe and I am MSc student in Medical Laboratory Science at Addis Ababa University. I am doing a research entitled "Performance Evaluation of Malaria Microscopists working at Malaria EQA Rechecking Laboratories in Ethiopia"**.**

The objective of the study is to assess the performance of malaria microscopists working at EQA Rechecking Laboratories. To assess the performance I have 12 validated blood film slides which will be examined by volunteer participants. So if you are agree to participate in the study, about 12 blood film slides will be given for examination and 10 minutes will be allocated for each blood film slides, and you will be interviewed. 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.

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

____

Subject's signature   date of signature

Survey Questionnaire

Questionnaire for "Performance Evaluation of Malaria Microscopists Working at Malaria EQA Rechecking Laboratories in Ethiopia," 2015.

Socio-demographic information

Questionnaire Number:___________________ Code of Health Facility: _________________

Interviewee Code:_______________________ Date of Interview:___________

1) Age (in years)________________

2) Sex

a) Male b) Female

3) Educational status

a) Diploma b) BSc

c) MSc and above d) Other Specify___________

e) Work Experience on Malaria Microscope

a. <2 year b) 2-5 Years c) > 5 Years

f) Types of Health Facility

a. RRL b) SRL c) HL d) HCL e) Other Specify---------

g) Are you full time employee?

a. Yes b) No

h) Have you taken Malaria Microscopy and QA training?

a. Yes b) No

i) An average BF slides Rechecked per person (by you)/per year___________

j) An average BF slides examined per person (by you)/per year (for Laboratories providing Routine Malaria Microscopy services)___________

Answer Sheet

Table A. Answer sheet for study participants

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. No	Slide ID							Parasite Density(Only for requested)	Remark
		Neg.	*Positive*				Stage of Malaria Parasite (for *positive* Slide)		
			PV	PF	PM	PO			
01									
02									
03									
04									
05									
06									
07									
08									
09									
10									
11									
12									
Total									

SOPs for Different Laboratory Procedures

SOP for Venous Blood Collection

Purpose

This SOP provides instructions for collection of fresh venous blood for malaria blood film (MBF) preparation.

Materials

1. Disposable syringes and needles or vacutainer tubes (EDTA/plain)
2. Tourniquet
3. Tube rack
4. Absorbent cotton wool/cotton
5. Alcohol (70% ethanol)
6. Disposable gloves
7. Clean glass slides
8. Pencil/pen/marker
9. Biohazard containers (for used needles/sharps and infectious waste)
10. Patient Register

Sample

Venous blood: obtained by vein-puncture; anticoagulant must be added to the blood to prevent the blood from clotting. Fresh non-anticoagulated or EDTA-anticoagulated blood should be used. Blood specimen should be obtained at the time of admission of the patient, irrespective of the periodicity of the fever. If these smears are negative, new smears should be made at various times midway between 6-12 hours after the next chill.

Procedure

Venous Blood Collection

Step	Action
1	Place a tourniquet above the venipuncture site.
2	Palpate and locate the vein. Disinfect the venipuncture site meticulously with alcohol by swabbing the skin concentrically from the center of the venipuncture site outwards. Let the disinfectant evaporate. Do not repalpate the vein again.
3	Perform venipuncture.
4	If withdrawing with conventional disposable syringes, withdraw 3-5 ml of whole blood.
5	If withdrawing with vacuum systems, withdraw the desired amount of blood directly into each transport tube.
6	Remove the tourniquet. Apply pressure to site until bleeding stops.
7	Using aseptic techniques, transfer the blood specimen to appropriate tubes (if using syringe). Secure tube caps tightly.
8	Label the tube with the patient’s unique identifier (name and/or number), date and time of collection, using a marker pen.
9	Do not recap used sharps. Discard directly into the sharps disposal container.
10	Complete the patient laboratory request forms using the same identifier.
11	Proceed to the preparation of malaria blood films.

SOP for Blood Film Preparation

Purpose

This SOP provides instructions for preparing good quality thick and thin malaria blood films.

Principle

The thick film is used as a screening test to establish the presence of malaria, and the thin film is used to identify the species of the organism. Examination of malaria blood films by microscopy is a basic technique, which remains the gold standard for the diagnosis of malaria. Good quality blood films are essential to establish accurate diagnosis.

Materials Supplies

1. Alcohol (70% ethanol)
2. Disposable gloves
3. Clean glass slides
4. Wooden block with grooves to hold slides
5. Pencil/pen/marker
6. Slide box/tray
7. Biohazard containers (for infectious waste)

Procedure

Using capillary blood:

Step	Action
1	Record the patient information in the appropriate form or register.
2	Label the frosted end of the slide with the patient ID/number and date.
3	From the pricked finger/earlobe/heel, collect blood directly in to the pre-labeled glass slides
4	Make both thick and thin blood films on the same slide as follows: By touching the slide on the blood, place a small drop of blood in the middle portion of the slide and 1 bigger drop on the portion next to the frosted end. Allow some space between the thick and thin films to be made on the same slide.

Using venous blood:

Step	Action
1	Using a micropipette, place a 2µl drop of blood in the smaller circle and 6 µl in the bigger circle of the slide (pre-labeled) placed over the template. Do not delay between applying and spreading the drop.

Preparation of the thin film

Step	Action
2	Working quickly, obtain a second clean and polished slide (spreader) and place it front of the small drop blood at a 30° - 45° angle. Pull back the slide and hold until the blood is evenly spread along the edge of the slide. Do not delay between applying and spreading the drop.
3	Rapidly push the slide forward in a single, smooth, continuous motion. Avoid hesitation or jerky motions when spreading the blood. (A feathered end of the film should have red blood cells that are lying individually

	without overlapping and relatively evenly distributed).

Preparation of thick blood film

Step	Action
1	With one corner of the spreader slide, in a circular motion, spread the blood out to make a circle with approximately **1cm (1/3 inch) in diameter,** finishing off at the center.
2	The ideal thickness of the smear should allow for printed text to be readable when it is placed on it.
3	Discard the spreader into an appropriate slide container and **DON'T** re-use it for another patient's blood sample.
4	Allow both the blood films to air dry in a horizontal position on a slide tray or folder. If EDTA blood is used, drying should be between 24–72 hours. Slow drying prevents cracking. Avoid using a fan or blow dryer to dry these slides.

Procedural Notes

A number of faults are common in making blood films. These can affect the labeling, the staining or the examination.

a) Badly positioned blood films

Care should be taken that the blood films are correctly sited on the slide. If they are not, it may be difficult to examine the thick film. Also, portions of the films may even be rubbed off during the staining or drying process.

b) Too much blood

After staining films made with too much blood, the background to the thick film will be too blue. There will be too many white blood cells per thick film field, and these could obscure or cover up any malaria parasites that are present. If the thin film is too thick, red blood cells will be on top of one another and it will be impossible to examine them properly after fixation.

c) Too little blood

If too little blood is used to make the films, there will not be enough white cells in the thick film field and you will not examine enough blood in the standard examination. The thin film may be too small for use as a label.

d) Edge of spreader slide chipped

When the edge of the spreader slide is chipped, the thin film spreads unevenly, is streaky and has many "tails". The spreading of the thick film may also be affected.

e) Thin film too big, thick film in the wrong place

If the thin film is too large, the thick film will be out of place and may be so near the edge of the slide that it cannot be seen through the microscope. During staining or drying, portions of the thick film will probably be scraped off by the edges of the staining trough or drying rack. It may be very difficult, or impossible, to position the thick film on the microscope stage so that it can be examined.

Quality Control

Monitor the quality of the preparation of thick and thin smears

1. Follow proper collection procedures.
2. Glass slides must be clean and free from grease.
3. Thick films and thin films must be prepared properly while drying protects blood films from dust, flies and insects.
4. Do not dry expressed to direct sun light.
5. Too thin a film may not have adequate quantity of blood for detection of parasite.
6. Blood film spread unevenly on a greasy slide makes examination difficult.
7. Thin film too long, leaves less space for thick film.
8. When fixing the thin film, be careful not to let methanol touch the thick film.
9. Wet slides are wrapped together and the slides stick to one another.

Figures

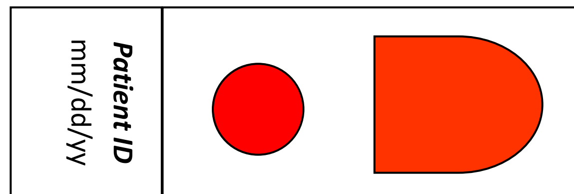


Figure A. Schematic representation of thick and thin malaria blood films

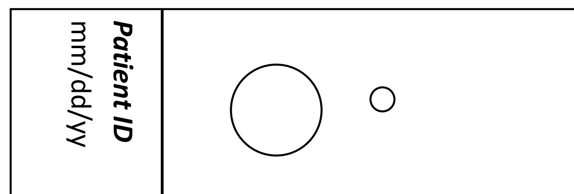
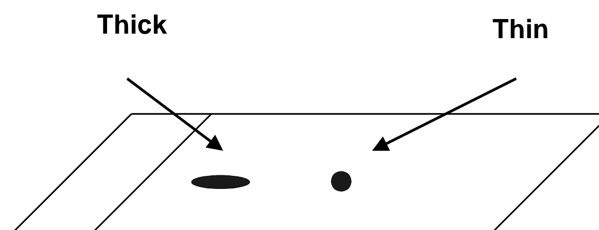


Figure B. Template for thick and thin malaria blood films



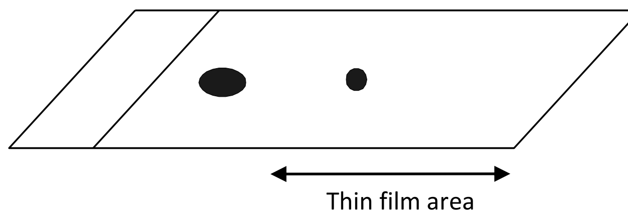
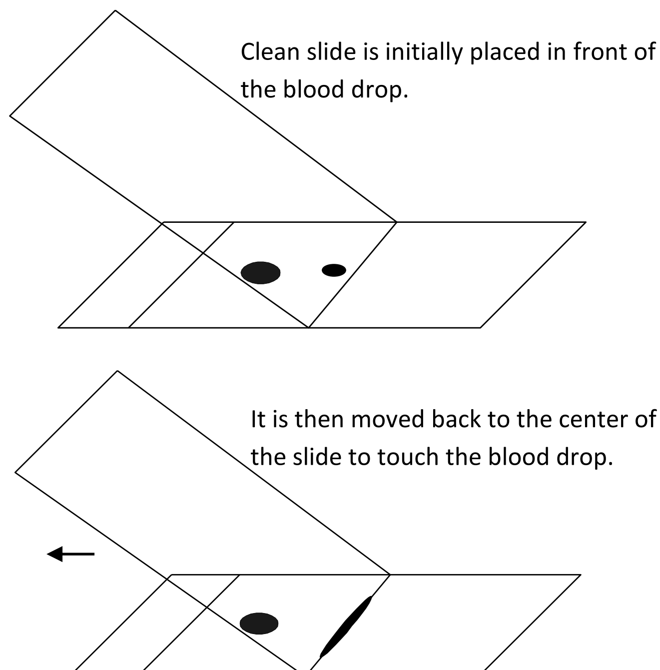


Figure C. Schematic representation for blood volume of thin and thick blood films

The larger blood drop on the left is for the thick film. The smaller drop represents what would be appropriate for a thin film.

The edge of a clean slide is placed at about 45° angle in front of the smaller blood drop for thin film. Slowly pull this second slide back into the drop while securing the sample slide with the forefingers of the other hand. Barely touch the drop of blood and, as the blood spreads laterally along at least two thirds of the edge of the "spreader" slide, rapidly push the spreader slide forward in a smooth, continuous and rapid motion, not stopping until the clean slide leaves the bloody slide. A properly prepared thin film is thick at the beginning end and thin or "feathered" at the other end. The feathered end of the smear should not reach to the end of the glass slide. The feathered end should have areas optimal for microscopy that are only one cell layer thick. The thin smear is best prepared immediately after applying the drop of blood, before any drying occurs.



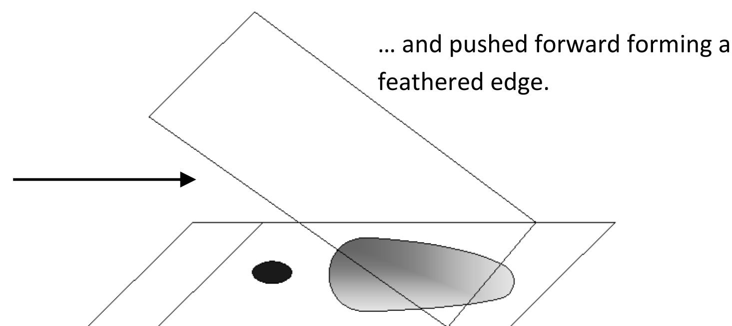


Figure D. Preparation of thin and thick blood films

The clean slide was placed just before the blood drop (to the right) then pulled back (to the left) and pushed forward to the right leaving a feather edged thin film. The blood for the thick film remains untouched at this stage.

Use the corner of the same clean slide to make the thick film by gently swirling the drop of blood to form an even circle of approximately **10 mm** diameter using the paper template over which the slide is placed during slide preparation. Once the drop(s) are evenly spread, lift the corner of the clean slide out of the center of the smear, trying not to leave any bubbles. If bubbles are present, stir again with the corner of the slide until no bubbles remain, and/or break the bubbles with the sharp corner of the spreading slide.

Allow the blood smears to dry in a horizontal position before storing it in a slide box. Blood smears should be left overnight (without applying any heat for rapid drying) before staining in order to obtain the best staining quality.

SOP for Staining

Purpose	This SOP provides instructions for proper staining of malaria blood films (thick and thin). Good quality staining of blood films is essential to establish accurate diagnosis.
Materials and and Reagents	Materials 1. Staining dish/jar 2. Drying rack 3. Wooden block with grooves to hold slides 4. Forceps 5. Gloves 6. Towels (paper) or sponge 7. Pencil/pen/marker 8. Patient Register Reagents 1. Absolute methanol 2. Dropper (with rubber bulb) 3. 3% or 10% Giemsa working solutions

Procedure

Fixing the thin film

Step	Action
1	When the films are completely dry, fix ONLY the thin film by dipping if in absolute methanol for approximately 30seconds. Care must be taken not to fix any portion of the thick film.
2	Allow the film to dry.

Staining the thick and thin films

Step	Action
1	Gently pour 3% or 10% Giemsa working solution in to the staining jar.
2	Put the slides in a rack inside the staining jar; the slides should be fully submerged/covered with the stain.
3	Stain for 30-45minutes and 10-15minutes for 3% and 10% Giemsa working solutions, respectively.
4	Pour clean water gently in to the jar to float off the iridescent scum on the surface of the stain. Alternatively, gently immerse the whole jar in a vessel filled with clean water.
5	Gently pour of the remaining stain, and rinse slides again in clean water for a few seconds. Pour the water off.
6	Wipe the back of each slide with paper towels.
7	Dry the slides in a vertical position with the thin film down wards.

Quality Control

Quality control of Giemsa stain is performed for every batch of stain prepared. QC results are documented using the form for *Quality Control for GiemsaStain.*

Preparation of control slides

1. To ensure that proper staining results have been achieved, a known positive smear should be included with each new batch of working Giemsa stain.
2. Control slides may be prepared from a patient's blood and stored for future use. From a patient known to have malaria infection, collect a blood film sample in an EDTA tube. An ideal blood sample has at least one parasite in every 2-3 fields on thin smears.
3. Make as many thin smears as possible, preferably within one hour of drawing the blood from the patient.
4. Allow the smear to dry quickly, using a fan or blower at room temperature.
5. Fix the smears in absolute (100%) methanol and allow them to dry.
6. Place them back to back in a slide box with separating grooves.
7. Label the box with the species, date and "Giemsa Control Slides".
8. The slides can be stored at room temperature but will last longer if stored at -70ºC.
9. Just before use, remove the slide from the box and allow the condensation to evaporate.
10. Label the slide with date and "+ control". The smear can be then be stained and examined to check that the working solution of Giemsa stain is good for quality.

Evaluation of a well-stained thin film

1. The background should be clean and free from debris; the color of erythrocytes is a pale green pink.
2. Neutrophil leukocytes have deep purple nuclei and well-defined granules.

3. The chromatin of malaria parasites is a deep purplish red and cytoplasm a clear purplish blue.
4. Stippling should show up as Schuffner's dots in erythrocytes containing *P. vivax* or *P. ovale*, and Maurer's spots in erythrocytes containing thelarger ring forms of *P. falciparum*.

Evaluation of a well-stained thick film

1. The background should be clean and free from debris, with a pale mottled-grey color derived from the lysed erythrocytes.
2. Leukocytes nuclei are a deep, rich purple.
3. Malaria parasites are well defined with deep-red chromatin and pale purplish blue cytoplasm. In *P. vivax* and *P. ovale* infections the presence of Schuffner's stippling in the "ghost" of the host erythrocyte can be seen especially at the edge of the film.

Evaluation of staining quality

1. A MBF that is too pinkish suggests low pH or over-staining.
2. A MBF that is too bluish or purplish suggests high pH or under-staining.

SOP for Cover Slipping (mounting) of Blood Films

Principle: Blood film for microscopic diagnosis of malaria can be made semi-permanent by cover slipping using appropriate mounting medium and cover glass. Mounted malaria blood films can be re-examined multiple times under oil immersion without causing damage to the smear or significant fading of the Giemsa stain.

Equipment and Material:

1. Mounting medium (e.g. Poly-Mount® of Polysciences , DPX mountant of PARK scientific limited, UK etc)

2. Cover glasses,

   a) Size 24 x 24 mm or 25 x 25 mm to cover paper LABEL.
   b) Size 24 x 50 mm or 25 x 50 mm to cover SPECIMEN.

3. Micropipette (1µl-100µl) and pipette tips

4. Small plastic droppers (to use to drop mounting medium)

PROCEDURES (stepwise):

For batch mounting, mounting medium should be transferred from its bottle container into a clean dropper, 1-2 mL at a time.

1. Make sure that the Giemsa stained blood films are completely dry.
2. Transfer ~~ 100 µL of mounting medium from the tube using micropipette and gently put it onto the specimen area of the slide, either by placing two drops (of about 50µL each) at approximately equal

distances from each other and from the edges of the specimen areas, or alternatively, the mounting medium can be applied to the slide along a single line approximately connecting the two drops shown in the illustration below. The technician should practice the techniques to determine which leads to the least bubble formation.

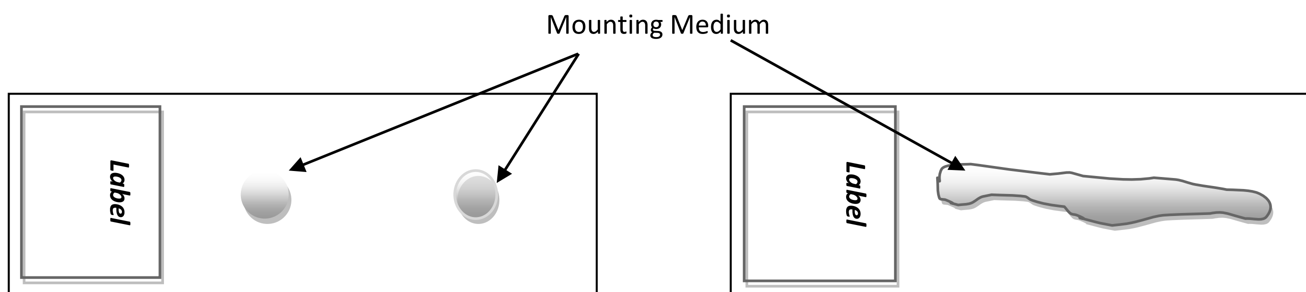
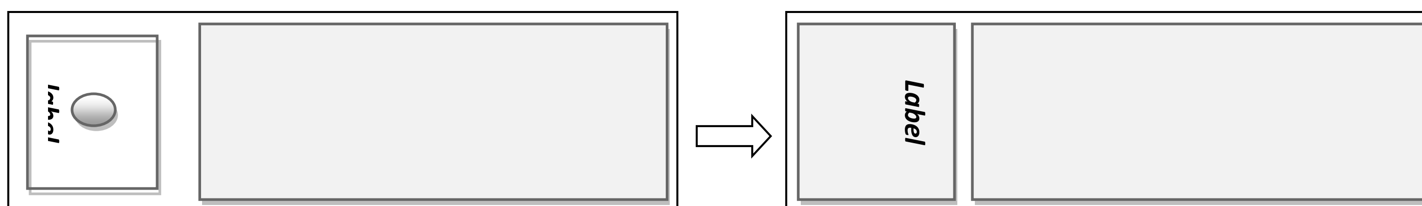


Figure E. Schematic Representation of blood film slide Mounting

3. Holding the cover slip by the edge in one hand, gently lower it to the surface of the slide, the first contact with the mounting medium should begin from one end of the cover slip in order to avoid any formation of air bubble.
4. Gently press on the top of the cover slip to allow the mounting medium drops to spread evenly across the slide and if necessary to remove air bubbles by putting slight pressure on the affected area without squeezing medium out from under the cover slip.
5. Wipe off any extra mounting medium from the slides.
6. Place drop of mounting medium directly on the paper label. Apply sufficient mounting medium so that when 24x24 or 25x25 label cover slip is applied the mountant completely covers (seals) the paper label.



Allow the mounted slides to dry overnight before examination or storage in boxes. Store slides in a light –free container.

Procedures Notes:

1. Work in an area with adequate ventilation and keep away from sparks and flame.
2. **Avoid sliding or asserting excessive pressure on the cover slip until completely dry.**
3. Following final labeling of slides, it has been found useful to protect the paper labels from immersion oil and other wear by covering them with square (24x24mm or 25x25mm) cover slips using mounting medium, as was done for the specimen itself.

Material Transfer Agreement

Ethiopian Science and Technology Commission, Health Department
National Health Research Ethics Review Committee
Address: Tel. 534945 P.O. Box 2490 Fax 251-1-524400
e-mail estc@ethionet.et Addis Ababa – Ethiopia

Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by Ethiopian Health and Nutrition Research Institute (EHNRI) and Hydas World Health in all transfer of blood material (dried blood spot and blood smears) related to the protocol.

Establishing Slide Bank to Improve Malaria Microscopy Quality Assurance Programs

Provider: Ethiopian Health and Nutrition Research Institute (EHNRI), Patriots Rd., Addis Ababa, Ethiopia

Recipient: Hydas World Health, 1814 Church Rd., Hummelstown PA 17036, USA

1. Provider agrees to transfer to recipient's designated **(Georganna G. Prescott)** the following materials **(Dried blood spot and blood smears).**

The patient material will only be used for the purposes as described in the protocol by recipient's investigator in designated laboratory for the project described below, under suitable containment conditions. This material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Project and the handling of the Research Material.

2. This material and its derivatives will be used by recipient's investigator solely in connection with the following project **(Establishing Slide Bank to Improve Malaria Microscopy Quality Assurance Programs in Ethiopia)** described with specificity as follows.

To confirm the parasite detection, species identification and enumeration of plasmodium parasite using species specific PCR and renowned slide validation experts

3. In all presentations or written publications concerning the project, recipient will seek agreement of provider and acknowledge provider's contribution of this material unless requested otherwise.

4. This material represents a significant contribution on the part of provider and is considered proprietary to provider. Recipient therefore agrees to retain control over this Material and further agrees not to transfer the Material to other people not under her/his direct supervision without advance written approval of provider. The dried whole blood will be disposed of as agreed upon per protocol at the end of molecular testing and completion of the project. However, the slides used in validation of the donations are considered as "Source Document". Therefore, for as long as the Ethiopian archive is functional the slides will be archived at HWH.
5. The provider does not take any responsibility for loss, damage, wastage or spoilage of the material during or after shipment to the address provided by the Recipient under conditions agreed to in the

protocol of shipment of the samples. This Material is provided as a service to the malaria prevention community. IT IS BEING SUPPLIED TO RECIPIANT WITH NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Provider makes no representations that the use of the material will not infringe any patent or proprietary right of third parties.

6. The recipient shall notify the provider in writing of any intention, improvement, modification discovery or development to the material or the information made by Recipient or parties, collaborating with Recipient, here in after referred to as “invention”. Nothing in this agreement shall, however, be construed as conveying to the provider any rights under any patents or other intellectual property to such invention, other than as explicitly provided herein. At its option the provider shall be entitled to receive sample of any materials derived from the Materials for its own evaluation purposes only.

7. The under- signed provider and Recipient expressly certify and affirm that the contents of any statements made herein are truthful and accurate.

8. Any additional terms (use an attached page if necessary):

9. The provider maintains, ownership right of the material and its derivatives unless stated otherwise.

Material Transfer Agreement Signature Page

For Recipient:

Recipient's Investigator Georganna G. Prescott, RN,MS Signature	Duly Authorized Signature/ Stamp
Date August, 2011 Mailing Address for Material:	Date August, 2011 Mailing Address for Notices:

Hydas World Health, 1814 Church Rd, Hummelstown PA 17036 USA

For Provider

Provider's Investigator Tilahun Muchie Signature	Duly Authorized Gudeta Tibesso Signature/ Stamp
 ------------------------------------ ------------------------------------	Gudeta Tibesso (MD+MSc) Director, Regional Laboratory Capacity Building Directorate ------------------------------------
Date: August 24, 2011 Mailing Address for Material: Ethiopian Health and Nutrition Research Institute Gullele Subcity, Patriots Road Addis Ababa, Ethiopia P.o.Box: 1242 Tel: +251 112 771052 Fax:+251 112 789121	Date: August 24, 2011 Mailing Address for Notices: P.o.Box: 1242 Tel: +251 112 771052 Fax: :+251 112 789121

Table for Rechecked BF Slides with Criteria for Good Blood Film Slides

Table B. Result table for rechecked BF slides

Code of Health Facility: ________________

Total number of slides stored/Quarter:______________________

Slide ID.	Result for Detection		Result for Species Identification		Slide Quality		Remark
	Site Result	Result of 2nd Reader	Site Result	Result of 2nd Reader	Good	Poor	
Total							

Slide Quality

1.Good

a.Thin film

i.Consist of a single layer of RBCs with feathered end

ii. Uniformly spread on the slide

b. Thick film

i. Round in shape,

ii. ~10mm away from the edge of the slide,

iii. Diameter ~10mm

iv. 10-12 WBC per single 100x objective field

2. **Poor** - do not fulfill one of the criteria listed above

Name and signature of 2nd Reader:______________________________

Name and signature of 3rd Reader: ______________________________

Date of Rechecking:______________

Check list for onsite supervision

Table C. Supervisory Checklist for Malaria EQA rechecking Laboratory

Facility Code___________________________

Tel. No_________________ **Fax**____________________________

Email_______________________

Conducted date _________________

I. General questions

No.	Questions	Comments
1.	Is the Laboratory Provide routine malaria smear microscopy service? a. Yes b. No If yes, how many BF smears are examined per year?------------------	
2.	Total No. Of staffs___ ______ Male____________ Female____________	
3.	Total No. Of staffs Participating on Blood Film Slides Rechecking______________	
4.	Total number of Junior Microscopists_____ Diploma__________ BSc__________ MSc and above__________	
5.	Number of laboratory personnel trained on malaria microscopy______________________ TOT__________ Basic_________	
6.	Is the Laboratory provided any training on malaria? a. Yes b. No If yes who were the participants? -------------------- When? ----------	

	For how long? ------------------------	
7.	Is parasitology Laboratory separated from other laboratory? a. Yes b. No	
8.	Is the laboratory using RDT for malaria diagnosis? a. Yes b. No	

II. Malaria microscopy laboratory format and supplies

	Are the following malaria microscopy formats and other materials available?	Items	1 = Available and being used 2 = Available, but not used 3 = Not Available	
		Malaria microscopy EQA guideline		
		Malaria Manual		
		SOP for malaria microscopy		
		Job aids		
		Laboratory Quality Manual		
	Are the following reagents and other Laboratory commodities available?	Item	1 = Available and being used 2 = Available, but not used 3= Not Available 4= Not Applicable	Enough for the coming 1 year 1= Yes 2=No
		Absolute methanol		
		Absorbent cotton wool		
		Beaker/volumetric flask		
		Brown bottle		
		Distilled water		
		Drying rack		
		Funnel		
		Giemsa powder/Giemsa stain stock solution		
		Glass beads		
		Glycerol		

		Immersion oil		
		PH Meter		
		PH Tablet		
		Filter paper		
		Timer		
		Lens paper		
		Measuring cylinder		
		Microscope slides		
		Glass-writing pen/lead pencil		
		Slide boxes		
		Staining rack		
		Staining jar		
		Tally counter		
		Tissue paper		
	Reagents labeled with its name, date of preparation and expiry date (observation)		1-Yes 2- No	

III. Equipment

	How many electric binocular microscopes do you have? --------------------	Brand name	# Functional	# Non Functional	Specific problem (examine stained blood film slide to fill this column)	Remark
		Total				

IV. Malaria microscopy skill assessment (when using for routine service)

	Which type of blood film do you prepare for Microscopy Examination?	1. Always thin smear 2. Always thick smear 3. As necessary 4. Always both (in the same slide) 5. Always both (separate slide)	
	How do you dry the film?	1.Air dry 2. Heat dry	
	Which part of the film (thin or thick) do you fix?	1.Thin 2. Thick 3. Both	
	Which type of staining method you are using?	1. 10% for 10 min 2. 3% for 45min 3. 3% for 30min	
	Do you perform quality control for giemsa stain	1. Yes 2. No If yes when and in how long period of time__________________ If no why_______________	
	Do you quantify *positive* results using one of quantification method (parasite density)?	1. Yes 2. No , If yes which method a. Number of parasite Against WBC b. Number of parasite Against RBC c. Percentage of infected RBC d. Semi quantitative(+ system)	

General comment of supervisor-
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____
____

Conducted by:______________________________

Signature:____________________ Date:________________

Criteria for Good Blood Film Staining

Evaluation of a well-stained thin film

2. The background should be clean and free from debris; the color of erythrocytes is a pale green pink.
3. Neutrophil leukocytes have deep purple nuclei and well-defined granules.
4. The chromatin of malaria parasites is a deep purplish red and cytoplasm a clear purplish blue.
5. Stippling should show up as Schuffner's dots in erythrocytes containing *P. vivax* or *P. ovale*, and Maurer's spots in erythrocytes containing thelarger ring forms of *P. falciparum*.
6. There should be no stain precipitate present on smear.
7. Stain should not be too dark or too pale.

Figure F. Schematic representation of good quality (unstained and stained) thin blood film slides

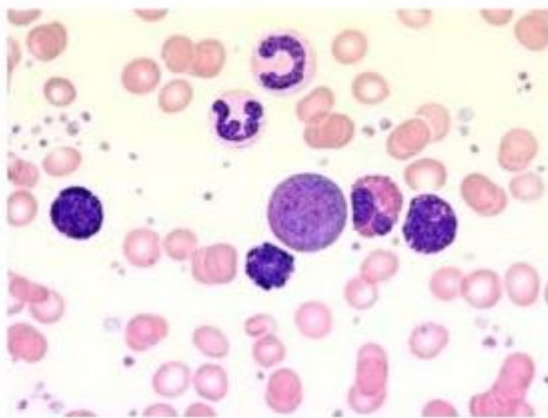

Figure G. Schematic representation of well stained thin blood film slides(Microscopic)