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Addis Ababa University

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Performance Evaluation of Laboratory Professionals on Malaria Microscopy among Health Facilities Found under Defense Health Main Department in Addis Ababa & Surrounding Area, Ethiopia.

BY: TIGIST YITBAREK

Advisor:

Mr. Kassu Desta (BSc, MSc, PhD Cand , Assistant Professor)

Mr. Biniyam Taye (BSc, MPH, PhD Cand , Assistant Professor)

A research thesis submitted to Department of Medical Laboratory Sciences, School of Graduate Studies, Addis Ababa University for the Partial fulfillment of the Degree of Master of science (MSc) in Clinical Laboratory Science with Specialty in Health Laboratory Management & Quality assurance.

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ADDIS ABABA, ETHIOPIA

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PERFORMANCE EVALUATION OF LABORATORY PROFESSIONALS ON MALARIA MICROSCOPY AMONG HEALTH FACILITIES FOUND UNDER DEFENSE HEALTH MAIN DEPARTMENT IN ADDIS ABABA & SURROUNDING AREA, ETHIOPIA.

BY: TIGIST YITBAREK

Approved by examining board

Chairman, Dep. graduate committee	Signature
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External examiner	Signature
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Internal examiner	Signature
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Advisor	Signature

Declaration

Assurance of Principal Investigator

I, the undersigned, conducted this research by adhering with all responsibilities for the scientific and ethical conduct of the research. I provided a timely progress report to my advisors and got the necessary advice and approval from them in the course of the research. I communicated timely to my advisors and all stakeholders involved in the study including any source of funding for this research.

Name of the student: Tigist Yitbarek (BSc)

Signature: _____ Date: _____/____/____

Place: Addis Ababa University School of Medical Laboratory Science, Ethiopia

Approval of the Advisors

This thesis has been submitted with my approval as university advisors.

Name of advisors: Kassu Desta (BSc, MPH, PhD Candidate , assistant professor)

Signature_____ Date___/___/_____

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Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University Approved By the Examining Board

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Advisor	Signature
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Examiner	Signature
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List of abbreviations

ACT	Artimesinin Based Combination Therapies
BF	Blood Film
EPHI	Ethiopia Public Health Institute
EQA	External Quality Assessment
FMOH	Federal Ministry Of Health
HC	Health Center
IRS	Indoor Residual Spray
MBFs	Malaria Blood Films
RDT	Rapid Diagnostic Test
SOP	Standard Operating Procedure
WBC	White Blood Cell
WHO	World Health Organization

Operational Definitions

Plasmodium:- is the genus of the parasite that cause malaria.

Malaria Microscopist:- is a professional laboratory person who uses a microscope to read blood films to aid or confirm the diagnosis of malaria and report their findings.

Performance:- is the skill of a malaria microscopists for performing an accurate examination and reporting of a malaria blood film.

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

Sensitivity:- is the probability of producing a true positive result when used in an

infected population as compared to a expert reader.

Specificity:- is the probability of producing a true negative result when used on a non infected population as determined by a expert reader.

In Training:- It is the lowest WHO classification on the performance of malaria microscopists. Personnel with this performance needs training to fill their gap in providing malaria microscopy services up to getting appropriate training.

Expert:- a person who has special skill or knowledge relating to a particular subject & those who were participated on doing and confirming the result of blood film slides.

Abstract

Background: Malaria diagnosis by microscopy is the gold standard method for confirming the cases. In spite of clinical diagnosis, the inability of laboratory professionals on microscopy diagnosis has led to over as well as under diagnosis. This may result in inappropriate use of anti-malarial drugs that increases the risk of drug resistance, and other diseases are overlooked and not treated in a timely manner.

Objective: To evaluate the performance of laboratory professionals in diagnosis of malaria species among selected health facilities found under Defense Health Main Department in Addis Ababa and surrounding area, Ethiopia.

Method: A cross sectional study was conducted from June to July, 2015. All laboratory professionals who work in selected health facilities were included in the study. Data was collected by distributing a standardized pre-validated malaria slide panel and self-administered questionnaires. Finally, data analysis was performed using SPSS version 20 to look an association between independent variable and dependent variable. An agreement in detection and species identification of malaria parasites between participants and expert microscopists was estimated using the Kappa score.

Result: The mean age of the participants was 32 ± 5.43 years. The majority of the participants 48 (80%) were males. Only 9(15%) participants used recommended quantification system. Five(8.3%) of the participants correctly reported all the distributed slides, whereas 55(91.7%) missed at least two slide. Overall, the sensitivity and specificity of performance among participants in detection of malaria parasites were 65.7% and 100% respectively. The overall agreement in performance between participants and reference readers on detection of malaria parasite was 71.4% (Kappa = 0.4) while on identification of malaria species, it was 51.1% (kappa = 0.04). Lower agreement was observed on detection and identification of slides with low parasitic density and mixed infection.

Conclusion: Agreement of performance among the participants and expert microscopist in the detection of malaria parasites was better than agreement in the identification of different species of malaria. Poor agreement was reported in detection of parasites at a low density and mixed infections. Hence, malaria control program should work towards increasing the competency of lab personnel in malaria microscopy, particularly in non-malarious area of the country where the professionals have lower exposure to malaria diagnosis.

Key words: Performance, Kappa test, Malaria, Blood Film

1. Introduction

1.1. Background

Malaria is a severe infectious disease caused by protozoan parasites: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malaria, Plasmodium ovale, and Plasmodium knowlesi* (1). *P falciparum* causes the most severe form of the disease accounting for 60% and tends to predominate in tropical areas. *P.vivax* is the predominant species outside Africa. In recent years, it has been increasingly recognized that *P.vivax* is also associated with severe symptoms (2). In the human body, parasites multiply in the liver, and then infect red blood cells. Symptoms of malaria include fever, headache, and vomiting, and usually appear between 10 and 15 days after the mosquito bite. If not treated, it can become life-threatening by disrupting the blood supply to vital organs (3).

Malaria is a serious public health problem in many parts of the world. Approximately, 86% of malaria deaths globally occurred among children under 5 years of age. According to the latest estimates, 198 million cases of malaria occurred globally in 2013 and the disease led to 584,000 deaths especially in children. Of these, 90% of all malaria deaths occur in sub-Saharan Africa (4). In Ethiopia, malaria become a leading public health problem where an estimated 68% of the population lives in malarious areas which comprises three-quarters of the total land mass(4). Its transmission varies with complex topography, ranges from high altitude mountain to low altitude fertile valleys. The main transmission season follow June to September rain, which is between September and December. Since the peak period of malaria transmission coincides with the peak periods of agricultural activity or coincids with the major harvesting seasons, results in a negative economic impact in the country (4, 5). Five main malaria eco-epidemiological strata are recognized in the country: 1) stable year round transmission in the western lowland and river basins areas of Gambella, 2) seasonal transmission in lowland areas below 1500 meters, 3) Epidemic-prone areas in highland fringes between 1500 & 2500 meters, 4) Arid area where malaria is found only near semi-permanent water bodies 5) Malaria-free highland areas above 2500 meters (4).

Early diagnoses of malaria leading to a prompt and effective treatment are the basis for the management of malaria and keys to reducing malaria mortality and morbidity (6). There are different malaria diagnosis methods in accordance with the level of health facility. Of those, the commons one are patient‘s clinical assessment, microscopic examination of blood slides and use of multi-species RDT. But, the detection of *Plasmodium* parasites by light microscopy is still the primary method of malaria diagnosis in most health care facilities throughout the world (7), and it has long been the method of choice for the diagnosis of many parasitic diseases, and until now it has also been the gold standard tool for malaria diagnosis. However, microscopy requires both technical skills and a power supply (8).

While light microscopy established over 100 years ago and frequently considered the reference standard for clinical diagnosis, it has been neglected in control programmes and evidences suggest field standards are commonly poor (9). 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 (10).

Malaria can be controlled through different way, vector control interventions have great role in contributed greatly to a reduction in the burden of the disease. Vector control measures should be repeated as necessary throughout the malaria transmission seasons. And, indoor residual spraying (IRS) is applicable as a long acting chemical insecticides on the wall & roofs of all houses and domestic animal shelter in a given area. Medicines and diagnostics also play a vital role for malaria management. Preferably medicine containing an artemisinin derivative therapy known as artemisinin-based combination therapies (ACTs). It is now considered that an effective first-line anti-malarial treatment would have a greater impact on reducing mortality (5,11, 12).

1.2. Statement of the Problem

High quality therapy of malaria is essential to avoid non-target effects, to delay the advent of resistance, and to save cost on alternative drugs. Confirmatory diagnosis before treatment initiation recently regained attention, partly influenced by the spread of drug resistance and thus the requirement of more expensive drugs unaffordable to resource-poor countries (13). On the other hand false positive and false negative results produce many consequences up to the death of the patient. In addition, misdiagnosis of malaria will result in the prescription of high cost drugs in the absence of the disease and exposure of potentially toxic drugs which may develop drug resistance (14, 15).

Low sensitivity and specificity of microscopic diagnosis of malaria leads to delayed treatment of malaria, development of serious complications, death or exposure to unnecessary treatment with other drugs (16). In practice poor microscopy has long been recognized and is a function of multiple factors, including training and skills maintenance, slide preparation techniques, workload, condition of the microscope, and quality of essential laboratory supplies (17).

Even among local laboratories with similar equipment and among reputed experts, abilities vary significantly. The microscopic detection of malaria requires technical skill, training, and maintenance of expertise and regular handling of samples containing the parasites. The quality of microscopy, however, often remains inadequate because of poor-quality equipment. A good smear has a role in reducing reading errors, whereas poorly prepared blood films generate artifacts that can be mistaken for malaria parasites (18).

Malaria laboratory diagnosis in Ethiopia is done by microscopists. The diagnosis is performed by general laboratory technologists assigned in health facilities where malaria diagnosis services are provided (19). The common complaint from the clinicians working in the city administration is that blood films are not as such supportive because of the usual negative results so that clinician working in Addis Ababa is primarily based on clinical judgment to treat patient. This problem is further compounded by the unavailability of studies to inform the real status of the laboratory professionals in the detection capacity of malaria smear microscopy in these areas and there is no previous study about evaluating the professions on malaria microscopy by distributing slide panel

which is stained and also unstained in Addis Ababa. However, some studies have been conducted in the area of evaluating the profession on malaria microscopy in our country Ethiopia.

As we compare to other part of Ethiopia, risk of malaria in Addis Ababa and surrounding area is law; because of that laboratory professionals are not exposed to malaria positive sample which brought its own impact on performance to malaria diagnosis with microscope. As Addis Ababa is a capital city, there are many people moving in and out. If someone is infected with mosquito its symptom is shown in 10 to15 days and when there is suppressive treatment just to prevent clinical symptom and paracitemia by destroying parasite in RBCs which is not prevent infection because of the parasite stage inoculate by mosquito (sporozoite) will survive and invade the liver & develop liver stage parasite and also when there is a recurrent infection it may be missed by professionals so as it is a matter of life studying performance of laboratory professionals will help to identify gaps in providing high quality microscopy based diagnosis of malaria and to inform decision makers and program managers.

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 (20) and there are many patients who are managed as malaria cases in spite of a negative blood film result that may be due to over suspiciousness of health workers or the perceived poor quality of laboratory finding (21).

Hence, this study aims to assess the abilities of professionals to detect & identify *Plasmodium* species on microscopy, and will point out the problems and gives information for further quality improvement in microscopic diagnosis of malaria in the so called non endemic places of Addis Ababa and surrounding.

1.3. Significance of the study

- This study provide information about the performance of malaria microscopists who are working on a non-malaria endemic area, like Addis Ababa & surrounding areas.
- This study findings reveal information which is useful to improve the performance of malaria microscopist based on the identified gaps.
- Provide information that, in which part malaria diagnosis(Detection, species identification & quantification) the microscopists had poor result
- It provides an information for policy developers, national and regional organizations, FMoH, and authorized bodies to work hard from their side based on the study findings.

2. Literature review

A study conducted in Ontario, Canada assessed the laboratory practice in the examination of blood films for malaria parasite. The result showed that there was shortcoming in the diagnosis of malaria parasite. All laboratories surveyed correctly identified the presence of malarial parasites in the blood film, but 27% misidentified the non-*falciparum* species as *P. falciparum* (22).

According to 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, the sensitivity and specificity were translated to 89% and 70%, 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 (10).

Retrospective survey conducted in Hong Kong for five years (2002-2006) assessed the quality of malaria diagnosis by distributing standardized malaria panels. In the assessment process by proficiency testing with unknown panels, more than 90% of participants achieved correct *p. falciparum* identification, but failed to give accurate parasitaemia estimation. In the assessment process by rechecking of routine slides, over all sensitivity and specificity were best in parasite detection but there was least agreement in mixed infection and *P. falciparum* identification(23).

According to a study conducted in Peruvian Amazon, a standardized set of 20 slides were used for the assessment of microscopists in diagnosing malaria. Of the 144 microscopists evaluated, 76 had more than one year of experience in microscopy and 64 had less than one year of experience. Microscopists with experience (68.6%) had more agreement than those without experience (48.2%). The competency assessment was found acceptable (competent, referent, or experts levels) in 11.8% of the microscopists without experience and in 52.6% from those with experience. The agreement was lower using blood smear slides with *P. falciparum* with low parasitemia, with *P. malariae* and with mixed infections. The case was higher among newly trained and inexperienced microscopists. (24)

A study conducted in Indonesia, 432 microscopists of 574 accepted invitations to participate in proficiency testing. Nearly all microscopists (413 or 95.6 %) scored at basic or in-training level, while 10 (2.3%) were advanced and 9 (2.1 %) were reference microscopists. Two thirds received at least one microscopy training during their career, with one third receiving no specific training (25).

A cross sectional study was conducted in Zambia, two of expert microscopists blindly reviewed 680 slides from six health centers (HC) after the HC technicians examined per his/her usual procedure. The HC technician and at least one of the expert microscopists agreed on the result of 144 positive slides (true positives) and 470 negative slides (true negatives). The two expert microscopists disagreed with the reading of the HC technician for 46 slides with a positive first reading (false positives) and 20 slides with a negative first reading (false negatives). The sensitivity was 88% and specificity was 91% (26).

A study conducted in Uganda in total of 184 of 192 (96%) identified laboratory personnel participated in the refresher training course. Pre-training didactic test scores were generally low, with an average score of 41%. After training, didactic test scores improved significantly, with an average score of 75%. A total of 1,079 thick blood smears were collected at selected health facilities before the training course, and 1,190 blood smears were collected approximately 1 month after the training course. Overall, sensitivity improved from 84% to 95% after training. The specificity of field microscopy (correctly reading a negative blood smear) ranged from 80% to 94% before training. After training, specificity ranged from 92% to 100%, with significant improvements in all four districts. Overall, specificity improved from 87% to 97% after training. The quality of blood-smear preparations improved dramatically after training. Across the four districts, the range of blood smears classified as good quality ranged from 4% to 8% before training and increased to 56–95% after training (27).

The study conducted in Republic of Congo, for all four slides were received from 263 participating laboratories; 92 (35%) reported a correct result on all slides. However, 3% reported no correct results and 11% reported only one correct result. 50.2% reported a correct result compared with 73.4 to 82.1% for the other slides. Major errors *Plasmodium falciparum* gametocytes (17.5%) and diagnosing malaria from the slide with no parasites (19%). The

frequency of serious errors in assessing parasite density and in reporting false-positive results was lower than in the previous external quality assessment: 17.2% and 52.3%, respectively, for parasite density and 19% and 33.3%, respectively, for false-positive results. laboratories that were participating in an external quality assessment for the second time did perform significantly better: 64 of 152 (42.1%) laboratories participating for the second time reported four correct results compared with 28 of 111 that were participating for the first time (25.2;) (28).

A cross sectional study conducted in Hawassa, Ethiopia shows from 72 participants, 14(19.4%) correctly interpreted all ten distributed slides, and 58(80.6%) missed at least one slide. Overall, the sensitivity and specificity of participants in detecting malaria parasites as compared to the two expert malaria microscopists were 82% and 96.5%, respectively. Agreement with expert microscopists was 88% on detection of malaria parasite. Participants also had 80.8% positive agreement for the six positive slides and 77.5% negative agreement for the four negative slides. Overall agreement between participants and the two expert malaria microscopists on detection and identification was 74.3%. Agreement in detection (88%) was much higher than in identification (74.3%) of malaria species. For both *Plasmodium vivax* and *Plasmodium falciparum*, worst agreement was found for slides with low parasite density (29).

A study conducted in north Gondar, Ethiopia showed a low agreement between health facility laboratory professionals and the reference reader. A total of 4,710 slides (2,355 slides read by operational readers and 2,355 unstained slides) were sent and examined for malaria parasites by the reference readers. overall specificity and positive predictive value were 73.7% and 58.1 % respectively which was considered as low. Agreement between the health facility laboratory professional readers and the reference was 75%. But the chance corrected agreement or kappa score was 0.47 (30).

3. Objectives

3.1. General objective

- To assess the performance of laboratory professionals in the diagnosis of malaria among health facilities found under Defense Health Main Department in Addis Ababa and surrounding area, Ethiopia.

3.2. Specific objectives

- To assess the ability of the laboratory personnel in detecting and confirming the presence of malaria parasites
- To assess the ability of laboratory professionals to differentiate species of malaria,
- To evaluate percent agreement between the laboratory technician/ technologist and the expert Microscopist

Hypothesis

I hypothesize that most malaria microscopists working at the study sites are in training level.

4. Materials and Method

4.1. Study area

The study was conducted in health facilities found under Defense Health Main Department of Addis Ababa and the geographical surrounding areas. Addis Ababa is the capital city of Ethiopia, which is situated in the foothills of the Entoto mountains and located at 2,400 meters above sea level. The city is an important administrative center not only for Ethiopia but also for the whole of Africa. As the capital city of Ethiopia, it serves as trade center where many migrant are coming to and fro as general. This study was conducted in health facilities found under defense health main department. There are hospitals which divided in three levels, level 1 hospitals, level 2 hospital, level 3 hospitals, armed force referral teaching hospital. Under those 16 health facilities found on average, there are 4 laboratory professionals per facility are found.

4.2. Study design and study period

A cross sectional study design was conducted from June to August, 2015 by distributing uniformly prepared stained and unstained malaria slides to evaluate the performance of laboratory professionals on malaria smear microscopy.

4.3. population

4.3.1. Source Population

The sources of population were all laboratory professionals who were working in health facilities found under defense health main department in Addis Ababa and surrounding area, Ethiopia.

4.3.2. Study population

The study population were all laboratory professionals who were working in health facilities found under Defense Health Main Department in Addis Ababa & surrounding area and who meet the inclusion criteria.

4.4. Eligibility

4.4.1. Inclusion criteria

All laboratory professionals who worked in selected health facilities who were present at the time of data collection

4.4.2. Exclusion criteria

All laboratory professionals who were on leave (sick, maternal) & who were not willing to give informed consent.

4.5. Sample size determination

All malaria microscopists who were working at study sites and those who were available during the study time were included in the study. There were a total of 60 malaria microscopists who were participated on the Study.

4.6. Sampling technique

Convenient sampling method was used. All laboratory professionals who work in health facilities and meet the inclusion criteria were included in this study.

4.7. Study variables

4.7.1. Dependent Variables

- Ability to detect *plasmodium* parasite
- Ability to identify *plasmodium* species
- Ability to Quantify *plasmodium* parasite

4.7.2. Independent Variables

- Educational level, Experience, age, sex, Training (Trained or not)

4.8. Data Collection process

4.8.1. Panel slide preparation and distribution

For the preparation of slides, five milliliters of whole blood was collected from acute febrile patients who attended Adama malaria control center. Blood was also collected from malaria negative persons that have no sign and symptom of malaria and who have no travel history to malaria endemic area for the past months. From those collected samples multiple set of slide panel were prepared which have high quality representing all common malaria parasite (*falciparum*, *vivax)*, various parasite densities (low, high), mixed infection and negative slides.

Two "level-one" microscopists who have been prequalified and certified by WHO and working in Adama malaria control center were involved in the preparation and as well as validation of malaria panels.

Both thick and thin blood films were prepared on a single slide. we were take 6 µl of blood (1drop) with automatic pipette and evenly spread on microscopic slide over an area of 11 x 12 mm and also we were taking 2 µl of blood for the preparation of thin blood film in each slide. After making the requisited number of slides, thin blood film was fixed with absolute methanol after being well dried and then both thin and thick were stained with 3% Giemsa working solution for 30-45 minutes. Finally, blood film for microscopic diagnosis of malaria was made semi-permanent by cover slipping using appropriate mounting medium and the cover glass and enough slides are left which is not stained. After preparation of panels completely the two expert microscopists arranged the slides in sets. During arranging the slides, they were verify the quality of slides and selected slides that have poor quality during preparation. Then, finally they validated those panels.

The two malaria microscopy experts interpreted the blood smears with 3 diagnostic keys: (1). the presence/absence of malaria parasite, (2) report the species parasites identified, (3) count the density of parasitemia for each species parasite count.

Quantification was performed on thick blood film against 200 white blood cells by taking the standard WBC count of 8000.

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

(4).

Slides were considered as negative if no malaria parasite seen in 100 oil immersion fields. After validation of slides completed, slides were arranged in sets and packed for distribution to the participant. The reporting formats, instruction letters and other additional information were packed separately.

4.8.2. Administering blood film slides

The malaria panels contained slides of standardized malaria blood smears with the following distribution: at least 1 slide of each species: stained negative slide (1slide), higher density *P. falciparum* (1slide), lower density *P. falciparum* (1 slide), higher density *P. vivax* (1 slide), lower density *P. vivax* (1 slide), mixed *P. falciparum* and *P. vivax* (1 slide) and the same number of slide which is not stained with a total of twelve slides.

Slide set (12 slides): Assessment of parasites detection, species identification & quantification

- 2- negative stained and unstained slides
- 2- *P. falciparum* stained and unstained slides of low densities
- 2- *P. falciparum* stained and unstained slides of high densities
- 2- *P. vivax* stained and unstained slides of low densities
- 2- *P. vivax* stained and unstained slides of high densities
- 2- Mixed species (including *P. falciparum* and *P. Vivax*) of stained and unstained slides

Based on WHO recommendation, quantification results of participants who 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(4).

4.8.3. Questionnaires

A structured questionnaires including information of the participating facilities and professionals were distributed. The socio-demographic characteristics, educational background and service, equipment like quality of microscope and slides, and training (in service) were collected.

4.9. Quality assurance

Data quality was ensured through use of standardized data collection materials, pretesting of the questionnaires, and intensive supervision during data collection by the principal investigator. Moreover, the panel slides were done following SOP

4.10. Data Analysis

Data was collected and entered into microsoft excel sheets and exported & analyzed using SPSS version 20 software. Association between levels of performance in identifying, detections, and quantification of malaria parasite was compared with independent variables collected by a structured questionnaire, and the result of the microscopic diagnosis of malaria reported by the participants was evaluated using various parameters. Mean, standard deviation, chi-square (for categorical data or to see the association between the performance and different socio demographic variables), sensitivity, specificity, and kappa score(to see the strength of an agreement) was calculated to assess the performance of the laboratory professionals. Based on WHO recommendation, microscopists were classified as: “In training”- when the agreement with the expert reader in detection & 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% (4).

Kappa Value was calculated 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 (31).

4.11. Ethical Consideration

The study was approved by the ethical clearance committee of Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences, departmental research and ethics review committee (DRERC). Official letters were written to the participating facilities. Consent forms was prepared in English to be read and signed (if agreed) by the participating health professionals and to blood donors. Interpreters were assigned for non-English speakers for donors. Participants were have the right not to participate in the study. A formal letter of cooperation was

obtained from armed force and referral teaching hospital. To ensure confidentiality, participants' data was linked to a code number only.

4.12. Dissemination of results

The findings of this thesis will be submitted to the department of Medical Laboratory Science, Addis Ababa University. It will also be disseminated to all stakeholders, public and concerned bodies through presentation in different professional association meetings and conferences in and outside the country. The summary result will be provided for health facilities as a feedback. The final paper will be sent to an international peer reviewing journal for publication.

5. Result

5.1. Socio-demography

A total of 60 laboratory professionals who were available during the study period participated in the study. Based on the data, the mean age of participant was 32 ± 5.43 years and about 48 (80%) participates were males (table 1). with the regard to educational background, among the respondents, 32(53.3%) and 28 (46.7%) were degree and diploma holders, respectively. Majority of the study participant such as 38 (63.3%) had an experience of more than two years & 22 (36.7%) were less than two year. of those study participants 20 (33.3%) were found to be trained on malaria microscopy; from these respondents all were trained at once. From those all participants, 36 (60%) participated in EQA program.

Table 1: Demographic characteristics of laboratory professionals working in health facilities found under defense health main department in Addis Ababa & surrounding area, Ethiopia (n = 60), 2015

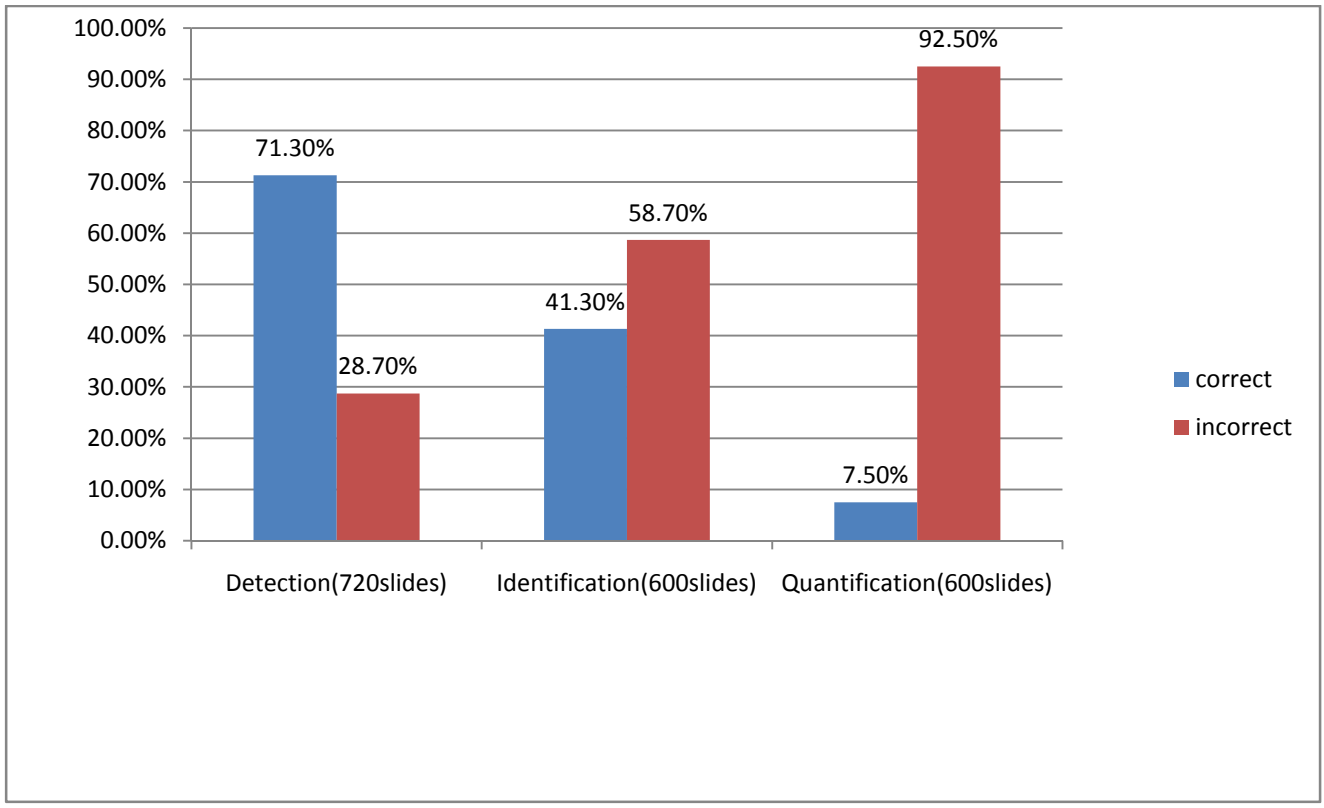
Variables	Number(n=60)	Percent (%)
Age (year)		
20-30	24	40.0
31-40	28	46.7
>40	8	13.3
Gender		
Male	48	80.0
Female	12	20.0
Educational Status		
Degree	32	53.3
Diploma	28	46.7
Experience in routine malaria diagnosis		
<2 years	22	36.7
≥2 years	38	63.3
Participation in in-service training on malaria microscopy		
Yes	20	33.3
No	40	66.7
Frequency of participation in malaria microscopy training		
Once	20	100.0
Twice	0	0.0
Does your lab participate in EQA program		
Yes	36	60.0
No	24	40.0

5.2. performance of laboratory personnel

Of the 60 study participants, only (8.3%) 5 correctly interpreted all distributed slides, and (91.6%) 55 missed at least two slides. From all 600 positive slides distributed, only 248 slides were correctly reported; and 100% of negative slides were reported correctly.Based on WHO grading system 40 (66.7%) of participants were in-training, 5 (8.3%) as advance, 10 (16.7%) as reference, & 5 (8.3%) as expert level in malaria parasite detection and 51 (85%) of all participant used un-recommended quantification system (table 2).

Table 2: Overall Performance of malaria microscopists on detection, species identification, stage identification and parasite quantification with their classification based on WHO recommendation, 2015

performance classification	**Frequency**	**Present**
percent agreement on Detection	**(n=60)**	**%**
≥ 90% (Expert)	5	8.3
≥ 80% (Reference)	10	16.7
≥ 70% (Advanced)	5	8.3
< 70% (In-training)	40	66.7
Total	60	100%
percent agreement on species identification		
≥ 90% (Expert)	5	8.3
≥ 80% (Reference)	0	0
≥ 70% (Advanced)	6	10
< 70% (In-training)	49	81.7
Total	60	100%
performance on parasite quantification		
Quantified both slides(All 10 positive slides)	9	15
used un recommended quantification system	51	85
Total	60	100%


100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
71.30%
28.70%
41.30%
58.70%
7.50%
92.50%
Detection(720slides)
Identification(600slides)
Quantification(600slides)
correct
incorrect

5.3. Sensitivity, specificity, and agreement in performance of malaria microscopists

Overall, the sensitivity and specificity of participants performance in detection of malaria parasites were 65.7% and 100%, respectively. The overall agreement on detection of malaria parasite was 71.4% (Kappa =0.4) which is 'fair agreement' (table 3)

Table 3: Over all sensitivity, specificity and agreement in performance of participants in detecting malaria parasite based on the total number of observations, 2015

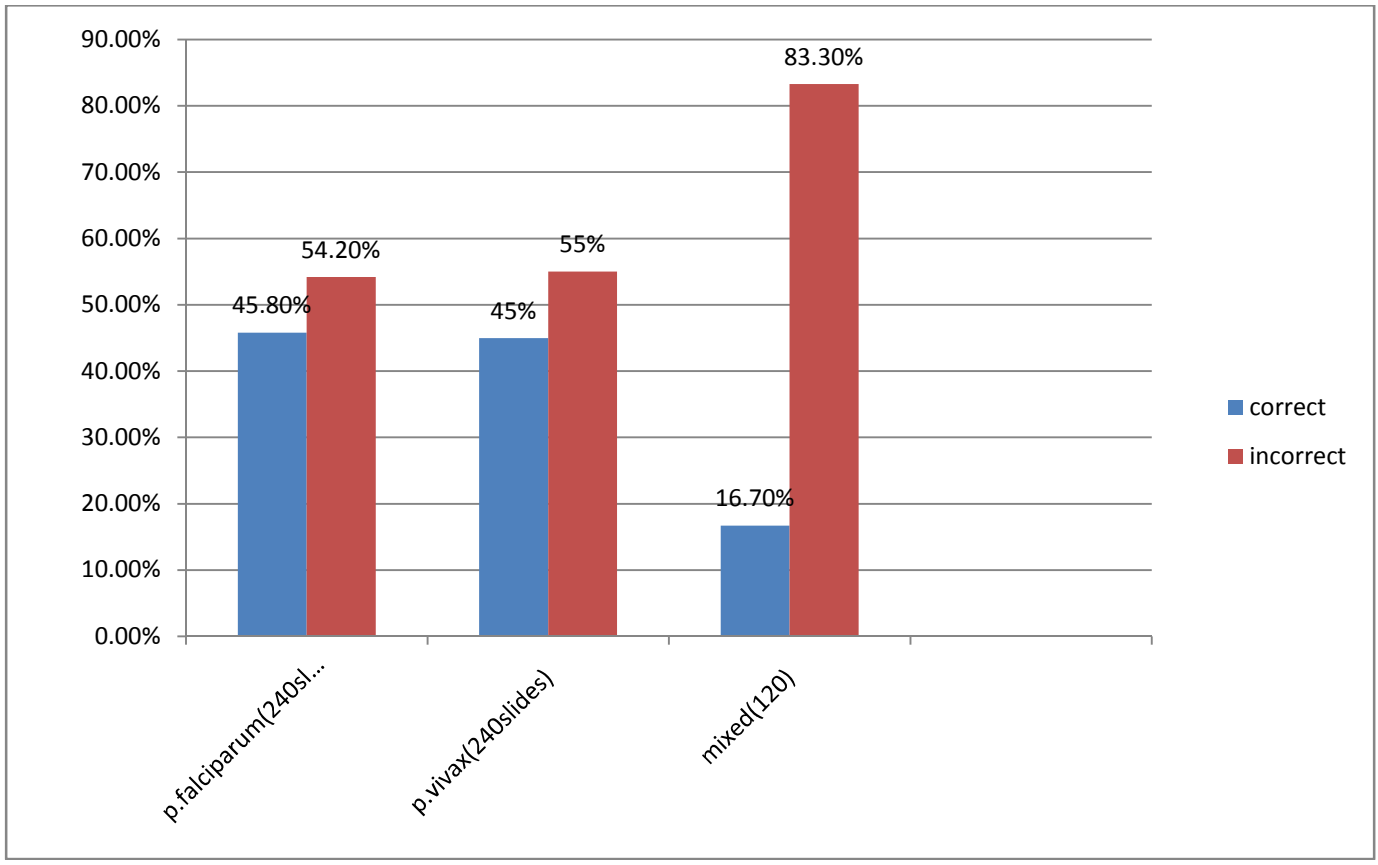
Participant Reader		**Expert Reader**			**Sensitivity**	**Specificity**	**Agreement**	**Kappa**
		Positive	**Negative**	**Total**				
Parasite Detection	Positive	394	0	394	65.7%	100%	71.4%	0.4
	Negative	206	120	326				
	Total	600	120	720				

Overall, the sensitivity and specificity of participants in species identification of malaria parasites were 41.3% and 100%, respectively (table 4). The overall agreement on identification of malaria species was 51.1% (kappa = 0.04) which is 'slight agreement'

Table 4: Over all sensitivity, specificity and agreement of participants in species identification of malaria parasite based on the total number of observations, 2015

Participant Reader		**Expert Reader**			**Sensitivity**	**Specificity**	**Agreement**	**Kappa**
		Expected Correct species	**Negative**	**Total**				
Species Identification	Reported species	248	0	248	41.3%	100%	51.1	0.04
	Negative	352	120	472				
	Total	600	120	720				

The performance of participants on species identification showed that 45.8% of BF slides with *P. falciparum*, 45% of BF slides with *P.vivax*, 16.6% of BF slides with mixed infection were identified correctly (fig 2). A total of 100 (83.3%) BF slides with mixed infection were identified wrongly as *P. falciparum*, 97 (40.4%) BF slides with *P. falciparum* were identified wrongly as negative, and 109 (45.4%) BF slides with *P. vivax* were identified wrongly as negative.


90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
45.80%
54.20%
45%
55%
16.70%
83.30%
p.falciparum(240sl...
p.vivax(240slides)
mixed(120)
correct
incorrect

From a total of 60 participants, the number of participants who scored< 80% higher among participants who had not malaria microscopy training.

Table 5: Relationship between some demographic characteristic with passed (≥80%) and failed (<80%) score on species identification based on WHO and national guideline (n=60)

Variables	Greater or Equal to 80%(Passed)	Less than 80%(Failed)	Chi-Square	p-value
Educational Status				
Diploma	2	26	0.097	.755
Degree	3	29		
Work Experience				
<2 Years	3	19	.496	.481
≥2 Years	4	34		
Participation on malaria microscopy and QA training				
Yes	3	17	1.745	.186
No	2	38		

There were a big difference in high & low density slides in terms of both for detection & species identification. The performance of participants on species identification showed that 75% of BF slides for *P. falciparum* high density for stained and 70% for unstained, for low density stained 25% of BF slides with *P. falciparum &* 13.3% for unstained.

Overall, the sensitivity and specificity of participants in detection of unstained slide with low density of malaria parasites were 17.5% and 100%, respectively. The overall agreement was 58.7% (Kappa = 0.17), which is almost 'slight agreement' (Table 6).

Table 6: **Over all sensitivity, specificity and agreement of participants in species detection of stained slide with high & low density and unstained slide with high & low density of malaria parasites based on the total number of observations**

Participant Reader		**Expert Reader**			**Sensitivity**	**Specificity**	**Agreement**	**Kappa**
		Expected Correct species	**Negative**	**Total**				
Detection stained slide with high density	Reported species	120	0	90	100%	100%	100%	1
	Negative	0	120	150				
	Total	120	120	240				
Detection stained slide with low density	Reported species	32	0	32	26.7%	100%	63.3%	0.26
	Negative	88	120	208				
	Total	120	120	240				
Participant Reader		Expert Reader			Sensitivity	Specificity	Agreement	Kappa
		Expected Correct species	**Negative**	**Total**				
Detection unstained slide with high density	Reported species	91	0	91	75.8%	100%	88%	0.76
	Negative	29	120	149				
	Total	120	120	240				
Detection unstained slide with low density	Reported species	21	0	21	17.5%	100%	58.7%	0.17
	Negative	99	120	219				
	Total	120	120	240				

From a total of 720 slides distributed half of which were unstained and the participant performance on detection & species identification of stained slides were better than the unstained ones in both detection & identification (table 7).

Table 7: Relationship between staining characteristics with Passed (≥80%) and Failed (<80%) score on species identification based on WHO and national guideline

Variables	Greater or Equal to 80%(Passed)	Less than 80%(Failed)	Chi-Square	p-value
Stained slides				
Detection	25	35	0.097	.755
Identification	12	48		
Unstained slides				
Detection	16	44	.496	.481
Identification	5	55		

6. Discussion

Malaria is a major public health problem in Ethiopia over the past years. Microscopy of Giemsa stained thick and thin blood films is the standard for the diagnosis of malaria. from a total participates, 42(60%) of them examine less than five slide per day and 44(63%) of the participants use both thick and thin blood films for detection and identification of malaria parasites but 16(23%) of participant were only examine thin films. There were no problems with the functionality of microscopes and accessibility of reagents in any of the laboratories and all laboratories where using Olympus microscope with binocular lenses. In the current study an agreement between participants and expert readers in the detection of malaria parasites was 71.4% and in identification of different species of malaria was 41.3%. only 5 (8.3%) participants were correctly interpreted all distributed slides, and 55(91.6%) missed at least two slides whereas study done in hawasssa 14(19.4%) participants were correctly interpreted all ten distributed slides,& 58(80%) missed at least one slides(29).

An overall agreement kappa value of this study finding on detection and identification of malaria parasites with expert readers was 0.4 which is classified as ‘Fair agreement’ and 0.04 which is classified as 'slight agreement' based on kappa index(31). The sensitivity and specificity of laboratory professionals in detecting malaria parasites were 65.7% and 100% respectively. The findings of low sensitivity were low in agreement and of high sensitivity with study conducted in Hawasa city, Ethiopia 82% & 96% (29), in Zambia 88% & 91%(26), in Uganda 92% & 87% (27) and in USA which was 92% and 90% (22). As compared to these study, our study shows lower sensitivity on the detection of parasites indicates that there were high false negative results which mean that there were high misdiagnoses of true infections. From a total of BF slides with mixed 100(83.3%)where identified wrongly as *P. falciparium,* the same study done in Hawassa city showed that most mixed cases were reported as *P. falciparum* (29) and there was least agreement in mixed infection in study done in Hong cong(23) and in the Peruvian Amazon where most cases of mixed infections were reported as negative or *P. vivax* (24). This is may be due to lack of awareness of the possibility of the presence of more than one species in blood films.

Nineteen seven (40.4%) BF slides with *P. falciparum* were identified wrongly as negative, and 109(45.4%) BF slides with *P. vivax* were identified wrongly as negative. The number of

participants failed to correctly speculate *P. falciparum* in the current finding was higher than the follow-up external quality assessment survey conducted in Canada 27% (21). This can lead to development of serious complication due to delay treatment and exposure to unnecessary treatment with other than anti-malaria drugs & lack of correctly identifying species may lead to incorrect administration of first line treatment. Correct species identification can be used to treat an individual with an appropriate first line drug and used to prevent drug resistance. the specificity in those three studies were above 90% including this study 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 may be due to they don't expect malaria parasite.

The overall agreement on detection of malaria parasite was 71.4% with Kappa of 0.4 which is defined as 'fair agreement'. Overall agreement in identification of malaria in the current study was 51.1% which was lower than the study conducted in Hawassa city with percent agreement of 74.3% (29). The strength of agreement in our study (kappa = 0.04) was lower than the finding reported in Hawassa city which is (Kappa=0.63) (29) and in North Gondar (kappa = 0.47) (30). this possibly due to lack of regular training or possibly they were not expect that malaria species were found in this area.

In our study 40 (66.7%) of participant were rated as 'in training' based on WHO recommendation while 17(23.6%) participants were rated as ‘in training” in other study in Hawassa city (29) there was 5(8.3%) of participant with expert level while study done in Hawassa city there were 18(25%) participants who were classified as expert & study conducted in Indonesia nearly all microscopic (95.6%) scored at basic or basic or in training level, while 10 were advanced & 9 were reference microscope (25). This may be due to lack of exposure to positive malaria blood film ,or lack of regular training on malaria microscopy.

From a total of 360 unstained slides 272(75.6%) BF slides were detected correctly which is somewhat lower than stained slides which is 232(64.4%) BF slides were detected correctly by participant and 168 unstained slides were identified correctly which is lower than 201 stained slides were identify correctly. poor staining quality may increase the number of incorrect result & from all slide distributed (600 slides of positive slides) only 248(41.3%) of them were reported correctly on species identification by participants which was lower than the study conducted in

USA which was 61% (10). The overall agreement on detection of malaria parasite stained slide with low density was 63.3% with Kappa of 0.26 which is lower than stained slide with high density which agreement of 100%.and from unstained slide with low density was 58.7% with kappa of 0.17 which is too lower than unstained slide with high density by agreement of 88% with kappa 0.76.

In this study from a total of participant only 9(15%) of them were quantify malaria parasite density. However, the majority 51(85%) of the participants were using semi quantitative system which were unrecommended quantification system. This was lower than the study done in Democratic Republic of Congo in which all of the participants used this quantification system (28). From those quantified slides, correctly quantified blood film slides were 50% which was lower than 81% of correctly quantified reported in USA (22). Lack of parasite quantification using recommended system may be due to lack of updated information, lack of training, 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(15).

7. Limitation and Strength of the Study

7.1. Strength of the study

- To the best of my knowledge this study was the first evidence based data in Addis Ababa.

7.2. Limitation of the study

- This study could not able to include all malaria microscopists who were working on study sites because they were not available during the time of data collection and some microscopists were resisting to participate.
- we were using only microscopic diagnosis of BF.
- This study did not use serology test (RDT) and molecular method Polymerase Chain reaction (PCR) to confirm parasite.
- This study also did not evaluate the performance of labratory professionals in identifying stage of plasmodium species.

8. Conclusion

- ✓ Poor agreement was reported in the detection of parasites at a low density level and mixed infection.
- ✓ Performance of participants were poor on detection & identification of malaria microscopy, their agreement with expert microscopist in the detection & identification of malaria species.
- ✓ Agreement of participants on quantification were very low. Many of laboratory technicians and technologist currently working at those laboratories has not taken sufficient training on malaria microscopy that is why high gaps were there between experts & participants and this has resulted in increase of significant gaps in profession lead to miss diagnosis.

9. Recommendation

Therefore,

- ✓ To fill those gaps all stakeholders have to work on the implementation of regular competency assessment and training policy.
- ✓ Demonstration of BF slides used for malaria microscopy training & have to evaluate the performance of laboratories using all external quality assessment methods and
- ✓ Have to conduct competency assessment for malaria microscopists for those who are working on study sites.
- ✓ Laboratories should practice on positive blood film slides regularly to improve their skill on detection & species identification.

10. Reference

1. Collins WE. "Plasmodium knowlesi: A malaria parasite of monkeys and humans". *Annual Review of Entomology*, 2012;57: 107–21.
2. Rogerson SJ, carter r: severe vivax malaria: newly recognized or re-discovered. Plos med 2008,5:136.
3. Ethiopian Health and Nutrition Research Institute (EHNRI): Malaria Laboratory Diagnosis External Quality Assessment Scheme Guideline. Addis Ababa, Ethiopia. 2009.
4. Ethiopian Health and Nutrition Research Institute (EHNRI): Manual for the laboratory diagnosis of malaria September, 2012 Addis Ababa, Ethiopia.
5. Federal Ministry of Health of Ethiopia (FMOH): National Malaria Guidelines, 3rd Ed, FMOH, Ethiopia, Addis Ababa, 2012.
6. World Health Organization. Bench aids for the diagnosis of malaria infections. Geneva, World Health Organization; 2002.
7. Pierre M, Philippe G, Albert L, Ben A, Simelo K, Jean L, et,al., External quality assessment of malaria microscopy in the Democratic Republic of the Congo. Malaria Journal 2011; 10:308.
8. Haditsch M: Quality and reliability of current malaria diagnostic methods. Travel Med Infect Dis 2004, 2(3–4):149–160.
9. Ashraf S, Kao A, Hugo C, Christophel EM, Fatunmbi B, Luchavez J. et.al. Developing standards for malaria microscopy: external competency assessment for malaria microscopists in the Asia-Pacific. Malar J. 2012; 24(11):352.
10. William R, Robert G, Martin P, Vernon M, Immo K, Joseph B.et.al., Performance of a malaria microscopy image analysis slide reading device. Malaria Journal 2012; 11:155.
11. Roll Back Malaria Partnership: Global Malaria Action Plan, 2008. http://www.rollbackmalaria.org/gmap/.pdf[Accessed on December 19, 2014]
12. Roll Back Malaria Department: WHO: Malaria Control Today, Current WHO Recommendations, WHO, Switzerland, Geneva, 2005.

13. Dicko A, Mantel C, Kouriba B, Sagara I, Thera MA, Doumbia S, et al. Season, fever prevalence and pyrogenic threshold for malaria disease definition in an endemic area of Mali. Trop Med Int Health 2005; 10: 550–556.
14. Barnish G, Bates I, Iboro J, Newer drug combinations for malaria. BMJ 2004; 328*:* 1511–1512.
15. WHO: Malaria Microscopy Quality Assurance, Version 1, WHO, Switzerland, Geneva, 2009
16. MalERA Consultative Group on Diagnoses and Diagnostics. A research agenda for malaria eradication: diagnoses and diagnostics. PLoS Med. 2011; 8: e1000396 17.
17. Wilson ML. "Malaria rapid diagnostic tests". Clinical Infectious Diseases 2012; 54 (11): 1637–41.
18. Houwen B. Blood film preparation and staining procedures. Clin Lab Med. 2002; 22:1–14. doi: 10.1016/S0272-2712(03)00064-7.
19. Abreha T, Alemayehu B, Tadesse Y, Gebresillassie S, Tadesse A, Demeke L,et al - Malaria diagnostic capacity in health facilities in Ethiopia 2011. Malaria J 2014, 13:292
20. Oliveira FJ, Lacerda MG, Brazil P, Ladislau JB, Tauil PL, Daniel RT. Malaria in Brazil: an overview. Malar J 2010; 9:115.
21. Kassahun M. Pattern of severe and complicated malaria in children admitted to GCMS hospital pediatrics ward. Ethiop. J. Health Dev. 2002;16 (1): 53-59.
22. Thomson s, Lohmann RC, Crawford L, Dubash R, Richardson H. External Quality Assessment in the Examination of Blood Films for Malarial Parasites within Ontario, Canada. Arch Pathol Lab Med. 2000; 124:57–60.
23. Leung WL, Kam KM. Malaria parasite quality assurance program in Hong Kong, 2002-2006. Journal of Hong Kong institute of medical laboratory sciences. 2007/2008; 11:8-15.
24. Rosas-Aguirre Á, Gamboa D, Rodriguez H, Llanos-Z F, Aguirre K, Llanos-CA. Use of standardized blood smear slide sets for competency assessment in the malaria microscopic diagnosis in the peruvian amazon . 2010; 27(4):540-7.
25. Lenny L. Ekawati1, Herdiana Herdiana, Maria E. Sumiwi,et.al. A comprehensive assessment of the malaria microscopy system of Aceh, Indonesia, in preparation for malaria elimination. Malaria J 2015:14:240

26. Lawrence B, James C, Margarette K, and Thomas S. Dose the availability of blood slide microscopy for malaria at health centers improve the management of person with fever in Zambia? Am.J.Trop.Med.Hyg.1999; 60(6):1024-1030
27. Moses Kiggundu , Samuel L. Nsobya , Moses R. Kamya , Scott Filler, et al, Evaluation of a Comprehensive Refresher Training Program in Malaria Microscopy Covering Four Districts of Uganda J. Trop. Med. Hyg, 2009 84(5), 2011, pp. 820–824
28. Mukadi P, Gillet P, Lukuka A et al., External quality assessment of malaria microscopy in the democratic republic of Congo. Malaria J, 2011; 10:308.
29. Ayalew F, Tilahun B & Taye,B Performance evaluation of laboratory professionals on malaria microscopy in Hawassa Town Southern Ethiopia, BMC research notes 2014,7:839.
30. Mitiku K, Mengistu G, Gelaw B. The reliability of blood film examination for malaria at the peripheral health unit. Ethiop. J.Health Dev, 2003; 17(3):197-204.
31. Anthony JV, Joanne MG. Understanding Interobserver Agreement: The Kappa Statistic. Research Series 2005; 37(5): 360-365.

11. Annexes

Annex I: Questionnaire

The information is collected by principal investigator.

Lab technicians/technologist code _____

serial	Question	Response Category	Remark
1	Age in Years	_____	
2	Sex	1. Male 2. Female	
3	When did you Graduate?	1. Less than 1yr 2. 1-2 years 3. More than 2 years_____	
4	Level of Education?	1. Diploma 2.BSC.	
5	Is the lab provide routine malaria smear microscopy service	1. yes 2. no	
6	How long has it been since you started working on malaria microscopy?	1. <2 years 2. ≥2 years	
7	How many malaria slides do you examine daily?	1. Less than 5 2. From 5-10 3. More than 10___	
8	Have you ever had an in-service training on malaria microscopy?	1. Yes 2. No	
9	If yes, for how long?	_____	
10	Which of microscope or RDT do u prefer	_____	
11	Does your lab. participate in EQA programs?	1.yes 2. no	

12	Is the staining solution are accessible and stored in appropriate place	1. Yes 2. No	
13	Is the microscope functional properly?	1. Yes 2. No	
14	What method do you use for the diagnosis of malaria	1- Thin film only 2- Thick film only 3- Thin and thick film	
15	Do you perform parasitemia?	1.yes 2.no	
16	If yes, which methods do you use	1- +, ++, +++ 2- Parasite per micro liter/ WBC 3- Parasite per micro liter/ RBC	

Annex II: Test procedure

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 for the purpose of positive slide and

simultaneously person who is known not to have malaria is also collected.

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 5-10 ml of whole blood from adults.
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 (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.

SOP for Blood Film Preparation

Purpose This SOP provides instructions for preparing good quality thick and thin malaria blood films (MBFs).

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

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 (See **Figure a)** 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 (See **Figure b)**.

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 See **Figure b** below). Do not delay between applying and spreading the drop.

Preparation of the thin film (See **Figure c, Illustration 1 and 2** below).

Step	Action
2	Working quickly, obtain a second clean and polished slide (spreader) and place it front of the small drop blood at a 30º - 45º 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 (See **Figure 3c, Illustration 3** below).

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

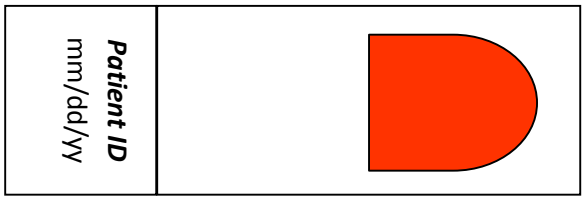


Figure A. Schematic representation Thick and Thin Malaria Blood Films

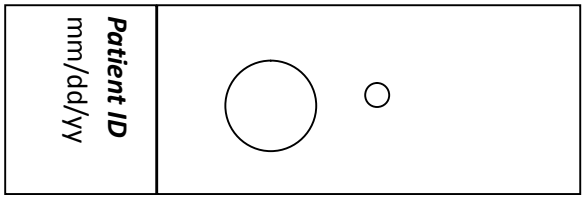


Figure B. Template for Thick and Thin Malaria Blood Films

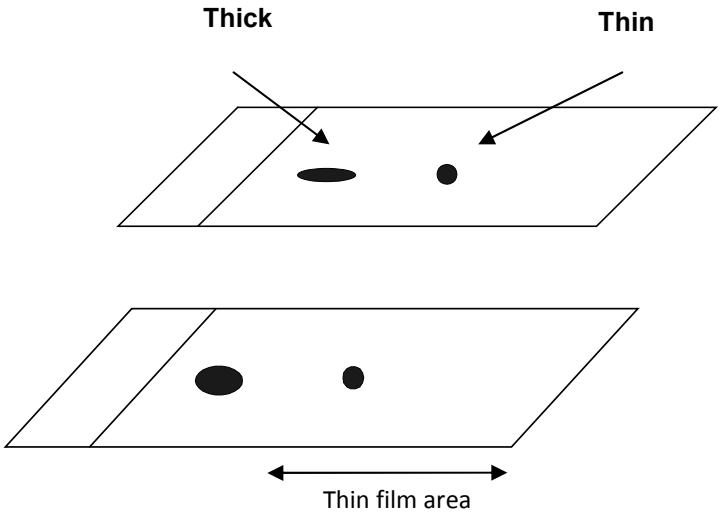


Figure C. Schematic representation for blood volume of thin and thick blood films

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 (see Illustration 2). 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.

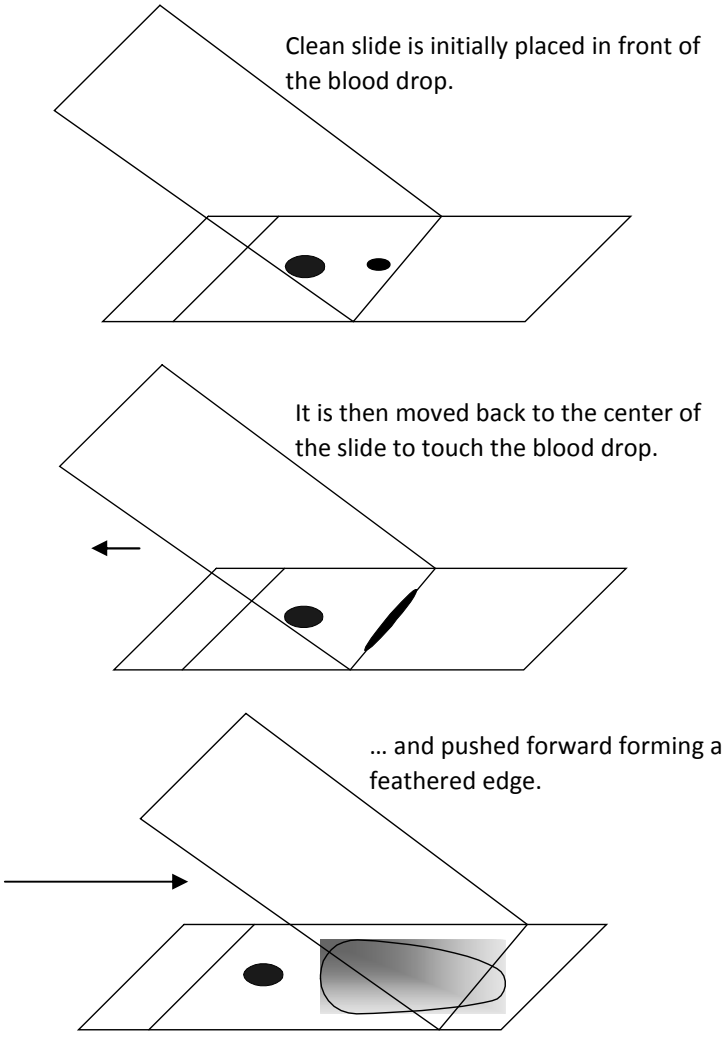


Figure D. Preparation of thin and thick blood films

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.

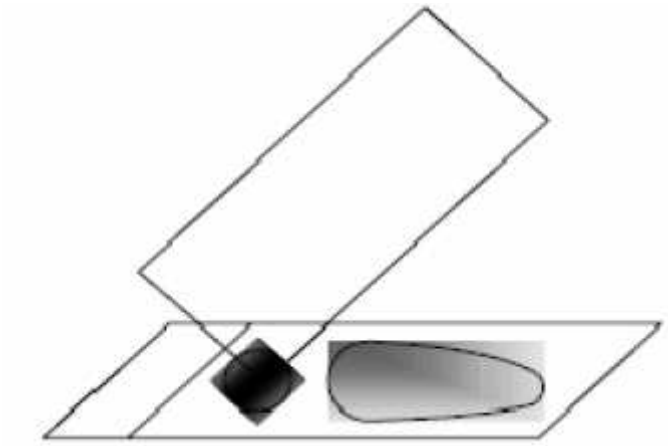


Illustration 3

Once the thin film area has been produced, use the corner of the clean slide to make the thick blood film

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.

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.

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.

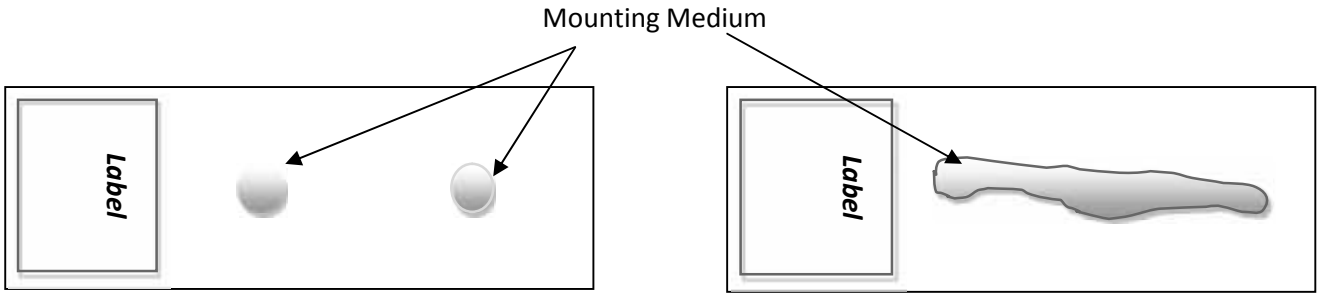


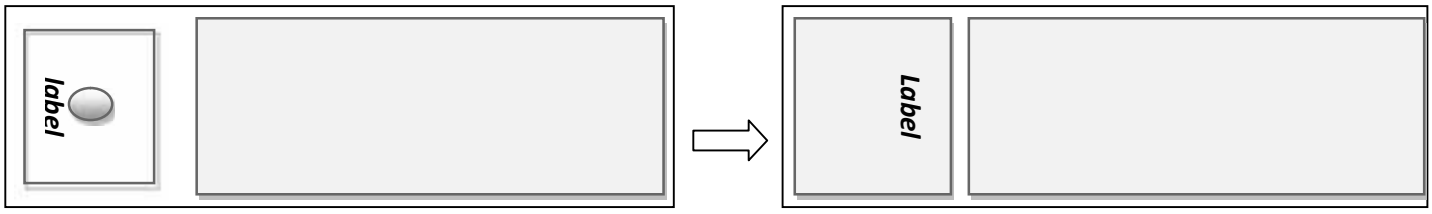
Figure 7.4.4. Schematic Representation of blood film slide Mounting

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.

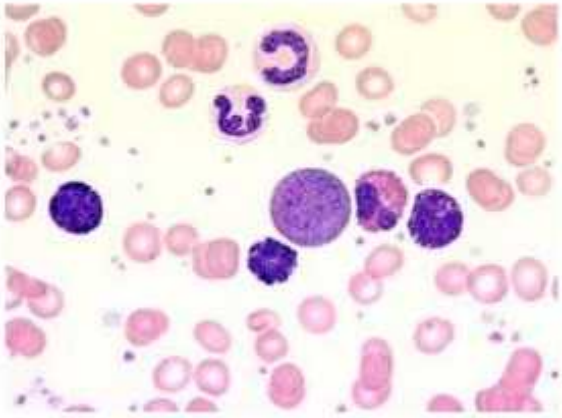
Procedures Notes:

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.

Criteria for good blood film staining

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* and Maurer's spots in erythrocytes containing the larger ring forms of *P. falciparum*.
5. There should be no stain precipitate present on smear.
6. Stain should not be too dark or too pale.



Annex III: Lab Result reporting format

Result reporting form for laboratory professionals

Lab technician ☐ Lab technologist ☐

Code Lab technicians/technologist _____

Slide ID	Result		
	Negative	Positive	
		Species	Parasite Load

Date: _____ Signature of the lab technicians/technologist_____

Annex III: Participant Information sheet and consent form

Background to the study

Malaria diagnosis by microscopical examination of blood smears is a method of choice which is the gold standard for confirming a clinical diagnosis of malaria. When a diagnosis is based on clinical symptom only and when there is inability of laboratory professionals to identify malaria parasites or to confirm its absence on blood smears has led to the inappropriate use of anti-malarial drugs for other febrile diseases, it associated with an increased risk of development drug resistance. it can also results Malaria can be over diagnosis considerably, while other diseases are overlooked and not treated in a timely manner.

Why the study is important

Evaluating the performance of laboratories in the diagnosis of malaria helps to identify any gaps which leads misdiagnosis of malaria and also helps to know how to improve the performance of the laboratories.

What are the risks of participating in the study?

There is no risk related to participating in the study.

Confidentiality

The information collected will not have any specific information including name that might break your anonymity.Any result given by the participants will serve only for this research not for any other purpose. You have the right not to participate in the study or can withdraw from the study any time. You are also not forced to tell anything you don't want to answer regarding yourself. If you have any question please contact PI

Tigist Yitbarek

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University Cell phone: +251- 09 12 079546 ;Email: tigisty2010@gmail.com

Consent form for malaria microscopist

ID. No: _____

This page contains an agreement signature to participate in the study entitled "Performance Evaluation of Laboratory professionals on Malaria Microscopy among Health Facilities at Defense Health Main Department Hospitals in Addis Ababa & surrounding area, Ethiopia."So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study.
2. I understand that, all the information given for the study and the results are confidential.
3. All the information is explained by supervisor.

Therefore, with full understanding of the situations I agree to give the entire necessary information and doing the procedure.

Signature of the participant: _____ Date: _____

Consent form for blood donors

This page contains an agreement signature to participate in the study entitled "Performance Evaluation of Laboratory professionals on Malaria Microscopy among Health Facilities at Defense Health Main Department Hospitals in Addis Ababa & surrounding area, Ethiopia." So please read the following points and sign your signature at the end in the space provided.

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