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Performance Evaluation of Laboratory Professionals on Malaria Smear Microscopy in Hawassa City, Southern Ethiopia

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Approved by the Examining board

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Examiner	Signature

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List of abbreviation/ acronyms

ACT	Artimesinin –based combination therapies
DRC	Democratic Republic of Congo
DRERC	Departmental Research and Ethics Review Committee
EQA	External quality assessment
IPTp	Intermittent preventive treatment for pregnant women
IRS	Indoor residual spray
LLIN	Long lasting insecticidal net
RDT	Rapid diagnostic test
RHB	Regional health bureau
SD	Standard deviation
SNNP	Southern Nations Nationalities and Peoples
WBC	White blood cell
WHO	World health organization

Abstract

Background: Microscopic diagnosis of Geimsa stained thick and thin blood films by skilled microscopists has remained the standard laboratory method for the diagnosis of malaria. However, diagnosis of malaria with this method is problematic since interpretation of results requires considerable expertise particularly at low parasite level.

Objective: The objective of the study was to evaluate the performance of laboratory professionals in diagnosis of malaria/plasmodium species in Hawassa city.

Methods: A cross-sectional study design was employed. Among a total of eighty laboratory professionals working in public and private health facilities, seventy two were willing to participate with a response rate of 90%(72/80). Information on demographic characteristics was collected using a self-administered questionnaire. A total of 10 pre-validated panels of malaria slides were then distributed to assess laboratory professionals' performances on detection, differentiation of common species of malaria and quantification of parasite density.

Results: The mean age of the participants was 27 (SD= 4.1) years and more than half of participants (56.9%) were female. Thirty-two (44.4%) of the participants were from two government hospitals, 9(12.5%) were from three private hospitals and 31(43.1%) were from six government health centers. Fourteen (19.4%) of participants correctly reported all the ten distributed slides and 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 and had 88% agreement with reference readers. The overall agreement between participants and reference readers for identification of malaria species was 74.3%; relatively the agreement was lower for government health centers (69%).

Conclusion: The overall sensitivity and specificity of participants in detection of malaria parasites were 82% and 96.5% respectively; however, they had low (74.3%) agreement in identification of different species of malaria. Especially, lower agreement was reported for parasites at low density and identification of mixed infection. Participants from government health center were found to have low performances in identification of malaria parasite.

1-Introduction

1.1- Background

Malaria is a serious infectious disease caused by a peripheral blood parasite of the genus Plasmodium. In human, it can be caused by one of five malaria parasites (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*), which have different geographical distributions. *Plasmodium falciparum* causes the most severe form of the disease and tends to predominate in tropical areas. *Plasmodium vivax* is the predominant species outside Africa. In recent years, it has been increasingly recognized that *P. vivax* is also associated with severe symptoms [1].

Malaria is a challenging, complex and deadly disease that potentially affects approximately 3.3 billion people in 109 countries and territories around the world. In 2000, there were between 350 and 500 million cases of malaria and at least one million deaths world-wide, most of them in African children. [2]. In addition to its health impacts, malaria places a heavy economic burden on many endemic countries, contributing to the cycle of poverty and limiting much of the economic development. For example, Africa alone is estimated to lose at least $ 12 billion per year in direct losses, and many times more than that is lost economic growth [2-4].

Ethiopia is one of the countries with an unstable transmission of malaria and most of its residential areas at risk. In Ethiopia, approximately 52 million people (68%) reside in malaria risk areas of altitudes below 2,000 meters. In this region, malaria is mainly seasonal with a more marked unstable transmission in the highland fringe areas and of relatively longer and more stable transmission in lowland areas, river basins and valleys. Approximately, there have been an estimated 10 million clinical malaria cases annually. Since 2006, however, the number of cases has reduced significantly. On average, 60%-70% of malaria cases have been caused by P. *falciparum*, with the remainder caused by P. *vivax*. *Anopheles arabiensis* is the main malaria vector; An. pharoensis, An. funestus and An. nili play a role as secondary vectors [5-7].

Malaria is the leading public health concern in SNNPR among the regional states of Ethiopia. Epidemiological and ecological data of the region indicate that more than 75% of the surface area of the region is malarious. Recent studies showed that areas as high as 2300 meters elevation have become prone to malaria outbreaks. Subsequently most areas of the region are affected by the disease. Malaria is the first cause of reported morbidity, and recently it has become also the first cause of hospital deaths [8].

Malaria control is a complex issue for various reasons. The war won in this regard can be lost any time if a small hole exists in the program/system. For instance, during the 1980s and 1990s, the burden of malaria increased in Africa as a result of drug and insecticide resistance and a general deterioration of primary health services [9].It also increased in intensity in the Eastern Mediterranean and South-East Asia sub-regions after the interruption of eradication efforts, and re-emerged in several central Asian countries as a significant threat to health. The emergence and spread of parasite resistance to previously effective low-cost drugs has, in particular, posed a major challenge for control efforts in all regions. The need to prevent both malaria infections and subsequent illness as well as to provide access to prompt treatment using newer combinations of effective drugs is ever more urgent [2, 4, 9].

Malaria can generally be prevented, diagnosed and treated with a combination of various methods. These tools may include long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and intermittent preventive treatment for pregnant women (IPTp) to prevent malaria infection in high transmission settings. Vector control measures (e.g. larviciding and environmental management) are also used as appropriate. Medicines and diagnostics also play a crucial role for malaria case management. Before drugs are prescribed diagnostics should make the diagnosis. Artimesinin-based combination therapies (ACTs) are the preferred treatment alternatives for P. *falciparum* malaria. Chloroquine (CQ) and primaquine (PQ) are the treatment of choice against chloroquine-sensitive P. *vivax* malaria [3, 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. Demonstration of the presence of malaria parasites prior to treatment with antimalarial drugs is fundamental to this goal, as clinical diagnosis has a poor accuracy and leads to over-diagnosis of malaria with resultant poor management of non-malarial acute febrile illnesses and wastage of antimalarial drugs. Moreover, this strategy increases the risk of antimalarial drug resistance [3, 10]. While microscopy remains the mainstay of parasite-based diagnosis in most large health clinics and hospitals, the quality of microscopy-based diagnosis is frequently inadequate for ensuring good health outcomes and optimal use of resources [3, 11].

1.2- Statement of the Problem

High-quality malaria diagnosis is important, because it improves patient care in parasite-positive patients as false negative results in untreated malaria patients potentially lead to death of the patients, and allows for the identification of parasite-negative patients in whom another diagnosis should be sought; it also reduces the use of antimalarials when there is no need, improves the accuracy of malaria surveillance, and provides confirmation of treatment failures. In addition, misdiagnosis of malaria will result in the prescription of high cost drugs in the absence of the disease and exposure of the patient to potentially toxic drugs which may develop drug resistance further emphasizing the importance of accurate microscopic diagnosis for malaria[11, 12] .

Clinical diagnosis of malaria is based on the patient's symptoms and on physical findings. The most prominent symptom is fever and it is often associated with chills, perspiration, anorexia, headaches, vomiting and malaise. These are often not specific and are also found in other diseases such as flu and viral infection. This results in considerable over diagnosis, particularly where malaria is seasonal [13].There are several methods devised to make a diagnosis of malaria in endemic areas. Parasite- based diagnosis is an important part of the case management of malaria, and WHO recommends that the demonstration of parasites should form the basis for treating malaria in all cases except among young children in areas of very high endemicity and during the control phase of malaria epidemics and emergencies [14].

Malaria microscopy is an old method introduced a century back by Ross [15]. Parasitic detection of the *Plasmodium* species is done by stained blood smears of malaria microscopy which is a simple, highly sensitive and low-cost method; and it is taken as the gold standard for malaria diagnosis. However, this method is time-consuming and interpretation of results is based on professional expertise especially in patients with low level parasitemia [16]. In many developing countries, microscopists are insufficiently trained, poorly supervised and burdened with high work load [17]. For instance, data from Tanzania showed low sensitivity and specificity of microscopic based diagnosis of malaria [18].

In Ethiopia, laboratory diagnosis of malaria used to be performed by malaria microscopists working in malaria control office. However, after decentralization and integration of an independent program, general laboratory technicians took responsibility for malaria diagnostics service in health facilities. However, the performance of these laboratory technicians in detection and identification of malaria are not known in Ethiopia, in particular, in the study area [19, 20].

Hence, this study aimed to evaluate the performance of laboratory professionals in identifying *Plasmodium* species using malaria microscopy. This study will point out the problems in this regard and it gives information for further quality improvement in microscopic diagnosis of malaria.

2-Significance of the study

Evaluating the performance of laboratory professionals in the diagnosis of malaria helps to identify area of improvement to provide high quality malaria diagnosis service. In practice, malaria diagnosis varies in different settings due to variable techniques used for blood film preparation, staining, blood film reading standards and skills of the examining microscopists. Poor quality blood film leads to false positive result due to the presence of artifacts commonly mistaken with parasites and inadequate staining leads false negative results. This is not only due to lack of provision of essential supplies like reagents and equipment and quality control system but also lack of skills of the laboratory professionals. There is no supervision and training to scale up professional performance. So this study is a good indicator of the need for in service training for laboratory professionals on malaria microscopy including regular national and regional supervision and internal and external quality control services.

Therefore, the current study evaluated the performance of laboratory professional in diagnosis of malaria microscopy which will be an important source of information for the stakeholders and quality officers to plan on job training and implement continuous supervision to improve microscopic diagnosis of malaria

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3-Literature Review

Various authors studied the performance of laboratories on the diagnosis of malaria. Study conducted in United States assessed the ability of laboratory professionals in diagnosis of malaria by examining the proficiency test (PT) results. The result showed that performance of the laboratories was best in detection and identification of *P. falciparum* (3.4% unacceptable responses), followed by *P. vivax* (9.0% unacceptable responses), *P .malariae* (10.9% unacceptable responses), and *P ovale* (20.6% unacceptable responses). Laboratories were most proficient at detecting and identifying *P falciparum* and substantially less proficient at identifying *P malariae*, *P vivax*, and *P ovale* [21].

3.1 Performance of laboratory professionals in detection

Study conducted in Haiti assessed the performance of health facility laboratory professionals in detection of malaria parasite. Of 207 smears identified as positive by health facility laboratory professionals, only 46 were confirmed. Health facility microscopists more accurately detect negative blood films, only 9 of 1261 were reported as false negative. In this study, health facility microscopists detected malaria parasite with a sensitivity 63.6% and specificity of 86.6% and they had 87.5% agreement in detection of malaria parasites [22]

In another study from Zambia, two of expert microscopists blindly reviewed 680 slides from six health centers after the health center technicians examined per his/her usual procedure. The health center (HC) technician and 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). Overall sensitivity was 88% and specificity was 91% and had 90.2% agreement with expert microscopists in detection of malaria parasite [23]

Another study conducted in Tanzania assessed accuracy of microscopic diagnosis of malaria and the result showed that, health facility laboratory professionals had 65% agreement with that of reference readers in detection of malaria parasite. The overall sensitivity and specificity of health facility professionals were 74.5% and 59% respectively. In this study, there were major variations in accuracy of malaria diagnosis between facility readers [24].

A study conducted in North Gondar Ethiopia showed a low sensitivity (78.5%) and specificity (73.7%) of health facility laboratory professionals on detection of malaria parasite. A total of 4,710 slides were sent and examined for malaria parasites by the reference readers. Health facility laboratory professionals had 75% Agreement in detection and 69% agreement on identification of malaria parasite with reference readers [25].

3.2- Performance of laboratory professionals in identification

A retrospective survey conducted in Hong Kong assessed the performance of laboratory professionals in diagnosis of malaria by distributing a standardized malaria panels. The result showed that blood film with *p.malarie* showed less than 50% of accuracy in differentiation among participating laboratories and there were only 5% of participants failed to report correct *P.falciparum* identification. There was also least agreement in mixed infection identification and no agreement in *p.ovale* identification [26].

A study conducted in Ontario, Canada assessed the performance of laboratory professionals in diagnosis of malaria by distributing three slides which contained three samples: *P.falciparum*, *p.vivax* and normal blood film. All laboratories surveyed correctly identified the presence of malarial parasites in the blood film, but 22% incorrectly identify *P. falciparum,* 2% of participant report parasite in normal blood film and one laboratory misidentify p.vivax. [27]

A study conducted in United Kingdom assessed the accuracy of routine laboratory diagnosis of malaria. Here from 17 *P. ovale* infections, only five were correctly diagnosed by submitting laboratories. Of 227 single species infections, 167 were correctly diagnosed. There were inaccuracy in identification of all species of malaria especially *P.ovale*. The most serious inaccuracy was that there was 21% failure rate for *P.falciparum* identification. Only one of the six mixed infections was accurately identifies and there was luck of awareness of the diagnostic possibility of mixed infection by the submitting laboratories. [28]

According to a study conducted in Peruvian Amazon, a standardized set of 20 slides were used for the assessment of microscopists for diagnosing malaria according to WHO guide line. Of the 144 microscopists evaluated 76 had more than one year of experience in microscopy and 64 had less than one year of experience. The result showed that the agreement of laboratory professional with the expert microscopists reading was lower using blood smear slides with P. falciparum with low parasitemia, with *P. malariae* and with mixed infections. These are being higher in newly trained and inexperienced microscopists [29].

3.3 Quality assessment in malaria diagnosis

A study conducted on external quality assessment of malaria diagnosis in the Democratic Republic of Congo revealed a poor quality of malaria diagnosis. They distributed panels which included three samples for diagnosis (sample 1: *P. falciparum*, high density, sample 2: *P. falciparum*, low density sample 3: no parasites seen), Among the participants, 26.2% scored all three samples correctly and 34.3%, 21.5% and 5.8% reported major errors in one, two or all three samples respectively. Major errors included missing the diagnosis of *P. falciparum* malaria and diagnosis of malaria in negative samples as well as errors in estimation of parasite density [30].

Again, a second study conducted one year later in the DRC showed that overall quality of Geimsa stained blood film microscopy was poor. The principal serious errors were: not recognizing *P. falciparum* gametocytes; diagnosing malaria from a slide with no parasites; and substantial quantitative errors in parasite density estimates. There were significantly fewer serious errors in the second external quality assessment than in the first. In the present quality assessment, 51 of 269 (19.0%) of the laboratories reported false-positive results for slides with no parasites, compared with 58 of 174 (33.3%) in the first assessment. Major errors in parasite density estimates were also less frequent in this assessment [31].

4-Objectives

3.1- General Objective:

To evaluate the performance of laboratory professionals in the diagnosis of malaria in Hawassa city

3.2- Specific Objectives:

- To determine the performance of the laboratory technicians/technologists on detection of malaria parasite in terms of sensitivity and specificity
- To determine the performance of laboratory professionals to differentiate common species of malaria.
- To determine the proportion of major and minor errors in diagnosis of malaria among participants

5-Methodology

4.1- Study design and study area

A cross sectional study design was conducted from November 1, 2013 to January, 2014 by distributing equal number of uniformly prepared malaria slides to evaluate the performance of laboratory professionals on detection of malaria smear microscopy in eight government and three private health facilities of Hawassa city administration. Hawassa is the capital of the Southern Nations Nationalities and Peoples Region (SNNPR) located at 275 km from Addis Ababa, the capital of Ethiopia. The altitude of the town is 1697m above sea level with mean annual temperature of 20.9 ^{0}C and a 997.6mm of rainfall. According to 2007 census conducted by the central statistical agency of Ethiopia, the total population of Hawassa town was 258,808 with almost 1:1 male to female ratio [32]. Hawassa is a malaria risk area and most of the time there are large number of people infected with malaria parasite especially during the month of August up to December.

In Hawassa city administration, there are two government, three private hospitals and six health centers. All these health facilities have laboratories that provide service to the patients including malaria microscopy. The total number of laboratory professionals found in these health facilities was eighty. According to the performance reports by the health facilities (obtained through a preliminary gathering of information directly from the candidate facilities by the investigator), on average, they saw five to ten slides per day.

5.2- Source population and sample size

The source population of this study consisted of all laboratory professionals working at eleven health facilities of Hawassa city. The number of laboratory professionals in these facilities was eighty. All the six government health centers, two government and three private hospitals that have laboratory professionals and provide laboratory service were included in the study.

From the total of eighty laboratory professionals, 72 were interested to fill out questionnaires and for slide panel test with a response rate of 90. Data were collected by distributing a standardized pre-validated malaria slide panel and self-administered questionnaires to assess the ability of laboratory professionals to detect malaria parasite in terms of sensitivity and specificity, differentiation of common species of malaria, correct reporting of parasite density and proportion of major and minor errors in diagnosis of malaria among study participants.

5.3- Study variables

Dependent- sensitivity, specificity, percent agreement, proportion of error

Independent – Educational level, age, sex, work experience

5.4- Data Collection process

5.4.1- Panel slide preparation and distribution

Four milliliters of whole blood was collected from acutely febrile patents that attended Adama malaria control center after obtaining informed consent and the blood samples were transferred in to separate EDTA containing glass tubes and processed within one hour of collection to preserve leucocyte and parasite morphology. Blood samples were also collected from malaria negative persons. The negative blood samples were pooled in to clean dry glass bottle for further use of negative slide set preparation. All cases positive for *P. falciparum* were treated with antipyretics and artemether-lumefantrine, whereas cases with *P. vivax* were treated with chloroquine and antipyretics [33].

Two expert microscopists who are pre-qualified and certified by WHO, work in Adama Malaria Control Center were involved in the preparation and validation of malaria panels.

In an attempt to standardize the preparation of thick blood films, we took 6 µl of blood with automatic pipette and evenly spread on microscopic slide over an area of 11 x 12 mm. This was done by placing the slide on a template marking the exact area. In addition, we also took 2 µl of blood for the preparation of thin blood film in each slide. Each slide with the thick and thin film were dried overnight and fixed by dipping in absolute methanol, avoiding contact between methanol and thick film and then, stained with 3% Geimsa for 30-45 minutes [34]. The blood films were mounted (by polymount) and cover slipped to increase its shelf life [35]. Once the Geimsa-stained slides become dry, they were stored in clean slide box.

After preparation of the panels was completed, the two expert microscopists arranged the slides in ten sets. Quality of slides was checked before packing the sets and the panels were finely validated before dispatching.

5.4.2- Slide panel characteristics

Slides were prepared based on the species present in the Region: *Plasmodium vivax*; *Plasmodium falciparum*; and mixed slides (*Pf/Pv*). Each panel of slides included:

- Slides with different parasite densities: low and high density: 6 slides.
- Negative slides: 4 slides.
- Total number of slides per panel: 10 slides

Groups of uniform panels with respect to the characteristics of the positive (species, and parasitemia) and negative slides were used so that the evaluation can be compared among the three laboratories.

The two malaria microscopy experts interpreted the blood smears towards 3 diagnostic keys:

1. The presence/absence of malaria parasite;
2. Identification of the species of parasites.
3. They counted parasitic load for each species parasite count per 200 white blood cell count in high power thick fields and multiplied by a standard multiplier of 8,000 WBC/µl of blood [11]

Slides were considered as negative if no malaria parasite was seen in 100 oil immersion fields. After validation of slides was completed, the slides were arranged in to 10 sets and were packed for distribution to the participant laboratories. The reporting formats and instruction letters were packed separately [36].

5.4.3- Administration of the Slide Panel Test

After validation, ten Geimsa stained thin and thick blood films and questionnaires were delivered to laboratory professionals working at 11 health facilities of Hawassa city. The panels included 10 slides for diagnosis, [slide 1: *P.falciparum* 104 /µl, slide 2: P.falciparum 53404/µl, slide 3 and 4: contain mixed (both *P.falciparum* and P.vivax), slide 5: p.vivax 23503/µl, slide 6: p.vivax 400/µl and slide 7, 8,9,10 were negative slides].

For examination purposes 10 minutes per slide were allocated [36]. Slide examination was performed by participants in two sessions: randomly, five slides of the panel of 10 slides were examined on the first day and the remaining five on the next day. Each of the participants spent 50 minutes per session on the day of their convenience. The data collector/ principal investigator retrieved the slides after each participant completed the tests.

5.4.4- Questionnaire:

A structured questionnaire including information of the participating facilities and professionals was distributed. The questionnaire had sub-components like: the socio-demographic characteristics, educational background, service, and their routine practice, type of equipment like the type and quality of microscope and slides, and training (in service). The questionnaire was first pre-tested on 5% of the participating professionals and was corrected as needed. (See Annex 2)

5.5- Data management and quality assurance

Data quality was assured through use of standardized data collection materials, pretesting of the questionnaires, and intensive supervision during data collection by the principal investigator.

5.6- Statistical Analysis

Data were entered, cleaned and analyzed using SPSS for windows version 20. Association between levels of performance in detection of malaria parasite was compared with independent variables collected by a structured questionnaire. Association between the outcome and the independent variables was taken as significant at $P<0.05$. Mean, standard deviation, chi-square (for categorical data), sensitivity, specificity, proportion of errors, percent agreement, kappa score were calculated to assess the performance of the laboratory professionals. Results were presented in tables and figures.

5.7- Judgment for participant performance

The results of the microscopic diagnosis of malaria reported by participants were evaluated by different parameters.

- Sensitivity- the ability of participants to diagnose positive blood films
- Specificity- the ability of participants to diagnose negative blood films
- Major error included (i) incorrect diagnosis of malaria, i.e. .reporting "negative" in the case of a Plasmodium-positive samples and vice versa, (ii) not mentioning the presence of *P.falciparum* (reporting non-falciparum species in the case of *Plasmodium falciparum*)[30].
- Minor error- false identification of p.vivax and mixed infection (reporting single infection in case of mixed parasite [26]
- The distinction between a minor and a major error was based on the potential effect the error could have on the patient's diagnosis and clinical management [31].
- According to WHO recommendations [13], participants were classified as a) "in training" when the agreement in detection of malaria parasite was less than 70% with the reference reader b) "competent" when the agreement was greater than or equal to 70% but less than 80%, c) "reference" when the agreement was greater than or equal to 80% but less 90%, and d) "expert" when the agreement was greater than or equal to 90% [13].

- Inter-rater agreement is the degree of agreement between two reference readers. It is calculated by computing the sum of true positives and true negatives and devises it by the total.

5.8- Ethical Consideration:

The study was approved by departmental research and ethics review committee (DRERC) of the Addis Ababa University, Department of Medical Laboratory Science. Official letters was written to the participating facilities. Consent forms were prepared in English to be read and signed (if agreed) by the participating health professionals and for blood donors . Information obtained about the laboratory professionals from the questionnaires, data capturing formats and the slides were totally kept anonymous. Participants had the right not to participate or withdraw from the study any time whenever they want.

6- Result

6.1 Socio demographic characteristics of study participants

A total of 72 out of 80 laboratory professionals responded to the questionnaires with a response rate of 90%. Thirty-two (44.4%) of the participants were from two government hospitals, 9(12.5%) were from three private hospitals and 31(43.1%) were from six government health centers. Of these, 19(26.4%) were generic BSc holders, 9(12.5%) were advance standing BSc and 44(61.1%) were graduates of diploma program. The mean age of the participants was 27 (SD= 4.1) years and more than half of participants (56.9%) were female. Among the participants, 56 (77.8%) had at least two or more years of experience in routine laboratory diagnosis of malaria and two third (68.1%) of the participants had never participated in formal training about malaria microscopy. For those who had a training, 78.3% had participated in a malaria microscopy training course only once during their practice. More than half (58.3%) of the participants reported that there was no supervision from regional or national laboratories and the vast majority of participants used microscopy for the diagnosis of malaria. Rapid diagnostic test was used only by 11% of the participants (Table 1).

Table 1-Demographic characteristics of participants participated in performance evaluation of malaria microscopic detection in Hawassa city, Southern Ethiopia

Variables	Number	%
Age in year		
20-30	59	89.1
31-40	12	16.7
>41	1	1.4
Sex		
Male	31	43.1
Female	41	56.9
Total	72	100
Place of work		
Government hospitals	32	44.4
Private hospitals	9	12.5
Government health centers	31	43.1
Educational status		
Diploma	44	61.1
Degree	19	26.4
Advance standing	9	12.5
Experience in routine malaria diagnosis		
<2 years	16	22.2
≥2 years	56	77.8
Participation in on- job training		
Yes	23	31.9
No	49	68.1
Frequency of participation in malaria microscopy training		
1 times	18	78.3
2 times	3	13
3 times	2	8.7
Have you ever supervised by regional or national laboratories		
Yes	30	41.7
No	42	58.3
Do you use RDT for malaria diagnosis		
Yes	8	11.1
no	64	88.9

6.2 Routine practice of participants in malaria microscopy

Participants were also asked about their routine practice for malaria diagnosis. Half of the participants stated that they examined more than ten blood film slides per day and 51(70.8%) of the participants used both thick and thin blood films for detection and identification of malaria parasites. On the other hand, a considerable number of participants (23.6%) examined thick films only. Regarding estimation of parasite density, more than half (56.9%) of the participants responded that they had experience on parasite count in positive slides and reported using grading system. All participants responded as no problem in the functionality of their microscope and accessibility of reagents (Table 2).

Table 2- Routine practice of participants in diagnosis of malaria parasites in eleven health facilities of Hawassa city, Southern Ethiopia

Variable	Number	%
Accessibility of staining solution		
Yes	72	100
No	-	
Functionality microscope		
Yes	72	100
No	0	
Number of slides examined per day		
<5	12	16.7
5-10	24	33.3
>10	36	50
Type of blood film used		
Thin film only	4	5.6
Thick film only	17	23.6
Thin and thick film	51	70.8
Parasitemia count		
Yes	41	56.9
No	31	43.1
Methods for reporting parasite density		
Semi quantitative(i.e. +, ++,+++)	41	56.6
Parasite/ µl in thick film against 200 WBC	-	-
parasite/ µl in thin film against 500 RBC	-	-

6.3- Status of laboratory professionals reporting on detection of malaria parasite.

Of 72 participants, 14(19.4%) correctly detected all ten distributed slides as proficiency testing panels and 58(80.6%) missed at least one slide. Eighteen (25%) of the participants reported correct results of all positive samples and 67(93.3%) of participants reported correctly all four negative slides (Table 3).

Table 3- Distribution of error in detection of malaria parasite reported by study participants in eleven health facility of Hawassa city, Southern Ethiopia

Variables	Number	%
All ten slides correctly reported	14	19.4
Only one slide missed	35	48.6
Two slides missed	19	26.4
Three slides missed	3	4.2
Five slides missed	1	1.4
All positive slides correctly reported	18	25
Only one positive slide missed	34	47.2
Two positive slide missed	19	26.4
Three positive slides missed	1	1.4
All negative slides correctly reported	67	93.3
One negative slide missed	2	2.8
Two negative slides missed	1	1.4
Three negative slides missed	1	1.4
All negative slides missed	1	1.4
Total	5	6.9
Parasitemia count	Not performed	

6.4 – Relationship between detection of malaria parasite with selected demographic back ground variables

Table 4- showed no significant association between errors made by participants in detection of malaria parasite and the selected demographic characteristics such as sex, experience, number of slides examined per day, in-service training, and supervision. (Table 4)

Table 4- Relationship between proportions of errors in detection of malaria with selected demographic characteristics of participants in Hawassa city, Southern Ethiopia

Variable	All ten slides	At least one error (%)	Chi squire	Degree of freedom	p-value
Sex					
Male	6(40)	25(43.9)	0.072	1	0.788
Female	9(60)	32(56.1)			
Experience					
<year's	3(18.8)	13(81.2)	0.205	2	0.903
≥2 years	11(78.2)	45(80.3)			
Number of slides Examined per day					
<5	3(20)	9(15.8)	0.752	2	0.685
5-10	6(40)	18(31.6)			
>10	6(40)	30(52.6)			
In service training					
Yes	3(20)	20(35.1)	1.243	1	0.265
No	12(80)	37(64.9)			
Supervision					
Yes	4(26.7)	26(45.6)	1.754	1	0.185
No	11(77.3)	31((54.4)			

6.5- Results of the microscopic diagnosis of malaria

6.5.1 Inter-rater agreement between two reference readers

Inter-rater agreement between two reference readers in detection of malaria parasite for each set was in strong agreement kappa= 1. (Table 5)

Table 5=Agreement of reference readers (RR1 and RR2) in detecting malaria parasites on n= 10 slides

		RR2**				
		Positive	Negative	Total	Agreement	Kappa
RR1*	Positive	6	4	10	100%	1
	Negative	0	0	0		
	Total	6	4	10		

RR1* = Reference reader 1

RR2**= Reference reader 2

Inter-rater agreement for identification of different species of malaria, the agreement between two reference readers were also perfect, kappa= 1(Table 6)

Table 6= Agreement of reference readers in identifying malaria species on n= 10 slides

		RR2**						
		P.falci	***P.vivax***	**Mixed**	**Negative**	**Total**	**Agreement**	**Kappa**
RR1*	***P.falci***	2	0	0	0	2	100%	1
	p.vivax	0	2	0	0	2		
	Mixed	0	0	2	0	2		
	Negative	0	0	0	4	4		
	Total	2	2	2	4	10		

RR1*= Reference Reader 1

RR2*= Reference Reader 2

6.5.2- Performance of laboratory professional in detection and identification of malaria parasite according to reference reader

Overall, the sensitivity and specificity of participants in detecting malaria parasite as compared to the two expert malaria microscopists reading were 82% and 96.5% respectively and had an agreement of 88% with expert microscopists reading. Participants also had 80.8% positive agreement for six positive slides and 77.5% negative agreement for four negative slides. Comparison across institutions showed lower sensitivity (81%) in those working in government health centers with an agreement of 87% with reference readers (Table 7 & 8).

Table 7= Sensitivity, specificity and agreement of participants in detection of malaria parasites based on 10 slides distributed to each health facilities (n= 72).

Malaria Parasite							
Participant	Reference reader						
Reader	Positive	Negative	Total	Sensitivity	Specificity	Agreement	Kappa
Positive	356	10	366	82%	96.5%	88%	0.76
Negative	76	278	354				
Total	432	288	720				

Table 8= Overall sensitivity, specificity and agreement of participants in detecting malaria parasite by health institution on 10 slides distributed to each health facilities (n=72).

Health institution	Participant reader	Reference reader						
		Positive	Negative	Total	Sensitivity	Specificity	Agreement	Kappa
Government hospitals	Positive	160	2	162	83%	98%	89%	0.78
	Negative	32	126	156				
	Total	192	128	320				
Private hospitals	Positive	46	3	49	85%	92%	88%	0.76
	Negative	8	33	41				
	Total	54	36	90				
Government health	Positive	150	5	155	81%	96%	87%	0.74
	Negative	36	119	155				

centers	Total	124	124	310

Observations were also made on agreement of identification of different species of malaria. The overall agreement between participants and the two expert malaria microscopists reading was 74.3%. Agreement in detection (88%) was much better than identification (74.3%) of malaria species. Comparison across institutions, it was even better in government hospitals (79%) as compared to private hospitals (74%) and government health centers (69%). It was the lowest in government health centers with an agreement of 69% with reference readers (Table 9 & 10).

Table 9= Overall agreement of participants in identifying malaria species on 10 slides distributed to eleven laboratories in Hawassa city (n=72).

Participant reader	Reference reader						
	Negative	*P.falciparum*	*P.vivax*	Mixed	Total	Agreement	Kappa
Negative	278	50	26	0	354	74.3%	0.63
P.falciparum	7	81	9	56	153		
P.vivax	3	11	109	21	144		
Mixed	0	2	0	67	69		
Total	288	144	144	144	720		

Table 10= Agreement of participants in identification of malaria species by health facilities on 10 slides distributed to eleven health facilities of Hawassa city (n=72).

Facility	Participant reader	Reference reader						
		Negative	*P.falci*	*P.vivax*	Mixed	Total	Agreement	Kappa
Government Hospitals	Negative	125	23	9	0	157	79%	0.70
	P.falci	1	35	1	25	62		
	P.vivax	1	4	54	1	60		
	Mixed	0	2	0	38	40		
	Total	127	64	64	64	319		
	Participant Reader	Reference reader						
		Negative	*P.falciparum*	*P.vivax*	Mixed	Total	Agreement	Kappa
Private Hospitals	Negative	33	6	2	0	41	74%	0.64
	P.falciparum	2	11	1	3	17		
	P.vivax	1	1	15	6	23		
	Mixed	0	2	0	9	11		
	Total	36	20	18	18	92		
	Participant reader	Reference reader						
		Negative	*P.falciparum*	*P.vivax*	Mixed	Total	Agreement	Kappa
Government Health Centers	Negative	119	21	15	0	155	69%	0.56
	P.falciparum	4	35	7	28	74		
	P.vivax	1	6	40	14	61		
	Mixed	0	0	0	20	20		
	Total	124	62	62	62	310		

6.6 Results regarding parasite density

Participants were asked to perform parasite count on each malaria positive slides using parasite /microliter against white blood cells in thick blood film. But the participants responded that they didn't even know how to count parasite density using these methods and totally unable to count parasitic load of malaria positive slides.

6.7-Errors reported by participants on each of 10 slides

The proportion of errors in detection and identification of different species of malaria found out that: 20.8% and 87.2% of participants reported correctly for *P.falciparum* positive slides 1 and 2 respectively; and 79.2% and 12.5% of participants reported major error for the respective slides. Similarly, 44.4% and 48.6% of participants reported correct results for slides 3 and 4 mixed infection respectively and 55.6% and 51.4% reported minor error. Regarding *P.vivax*, 88% and 61.1% of participants reported correctly for *P.vivax* slides 5 and 6 respectively. Whereas, 1.4% and 34.3% reported major error and 9.7% and 34.2% reported minor errors for slides 5 and 6 respectively. And for the four negative slides, majority of the participants reported correct results. Only a small proportion (1.4%, 2.8%, 4.2% and 5.6%) of participants made major error on the respective negative slides. (Table 11)

Table 11- Detection and identification errors made by participants on 10 slides distributed to each facility in Hawassa city, Southern Ethiopia (n= 72).

i. Positive slides					
	Correct (%)	Major error (%)			Minor error (%)
Slide no		Detection error (%)	Identification error for *P.falciparum* (%)	Total	Identification error for mixed and *P.vivax*
1	15(20.5)	48(66.7)	9(12.5)	57(79.2)	-
2	63(87.2)	2(2.8)	7(9.9)	9(12.5)	-
3	32(44.4)	-	-	-	40(55.6)
4	35(48.6)	-	-	-	37(51.4)
5	64(88)	1(1.4)	-	1(1.4)	7(9.7)
6	44(61.1)	25(34.3)	-	25(34.3)	3(4.2)
ii. Negative slides					
Slide no	Correct (%)	Detection error (%)	Identification error	Total (%)	Minor error
7	71(98.6)	1(1.4)	-	1(1.4)	-
8	70(97.2)	2(2.8)	-	2(2.8)	-
9	69(95.8)	3(4.2)	-	3(4.2)	-
10	68(94.4)	5(6.9)	-	5(6.9)	-

NB- slide 1& 2- *P.falciparum* positive, slide 3&4- mixed infection, slide 5&6- *P.vivax* positive slides and 7, 8, 9& 10 are negative blood films.

In total, 44.4% and 48.6% of participants reported correct results on slide 3 and 4 of mixed infection respectively. Most case of mixed infection was reported as *P.falciparum* by 36.1% of participants in slide 3 and 41.7% of participants in slide 4 (Table 12).

Table 12- Results for mixed infection slides reported by participants (n=72).

	Mixed slides	
Response	Slide 3	Slide 4
Mixed	32(44.4%)	35(48.6%)
P.falciparum	26(36.1%)	30(41.7%)
P.vivax	14(19.4%)	7(9.7%)

6.8 Classification participant performance based on agreement with reference reader

Regarding c classification of participant performance evaluated according to the WHO grading system, 17(23.6%) were rated as 'in training", 23(31.9%) considered as "competent", 14(19.4%) categorized as reference, and 18(25%) reached agreement on an expert. Among 56 participants who had two or more years of experience in microscopic diagnosis of malaria, 11(19.6%) were classified as 'in training', 18(32.1%) were competent and 13 (23.2%) were categorized as "reference" and 14(25%) reached agreement on an "expert". Regarding participants who had less than two years of experience, 6 (37.5%) were classified in the group of "in training", 5(31.3%) were considered" competent", 1(6.3%) achieved at reference and 4(25%) reached to expert (Table 13).

Table 13- classification of participant performance in detection and identification of malaria parasite based on experience

Classification based on agreement with reference readers	Participant < 2 years' experience		Participants ≥ 2 years' experience		Total	
	N= 16	%	N= 56	%	N=72	%
In training	6	(37.5)	11	19.6	17	(23.6)
Competent	5	(31.1)	18	32.1	23	(32)
Reference	1	(6.3)	13	23.2	14	(19.4)
Expert	4	(25)	14	25	18	(25)

Among participants who had two or more years of experience, 94.6% of participants had agreement for negative blood films, 64.3% and 23.2% of participants had agreement for *P. vivax* and *P.falciparum* slides with low parasite density respectively according to the parasite count reported on each parasite positive slides by the two expert microscopists and 35.7% for mixed infection. Regarding participant who had less than two years of experience, 87.5% of participants had agreement for negative blood films, 50% and 12.5% of participants had agreement for *P. vivax* and *P.falciparum* with low parasite density respectively and 25% for mixed infection. (Table 14)

Table 14- Agreement of participants with reference readers for negative and positive slides based on experience.

	agreement of participants	
Slide characteristics	Participants with 2 years' experience	Participants with 2 or more years' experience
Negative slides	87.5%	94.6%
P.vivax at low density	50%	64.3%
P.falciparum at low density	12.5%	23.2%
Mixed	25%	37.7%

7-Discussion

Microscopy of Geimsa stained thick and thin blood films are standards for the diagnosis of malaria; however, interpretation of results requires professional expertise especially parasites at low density. In the current study, agreement between reference readers and participant on detection of malaria parasite was 88% and in identification of different species of malaria was 74.3% (agreement with reference readers). Fourteen (19.4%) of participants correctly reported all the ten distributed slides and 58(80.6%) missed at least one slide.

Overall, the sensitivity and specificity of laboratory professionals in detecting malaria parasites were 82% and 96.5% respectively. These findings of low sensitivity and relatively high specificity were in comparable with study conducted in Zambia where the sensitivity and specificity of laboratory professionals were (88% and 97%) in Zambia [23]. This low sensitivity in detection of malaria parasite may indicate the possibility of false negative results which may result in delayed treatment for malaria, development of serious complications and death or exposure to unnecessary treatment with other drugs. However, our findings of sensitivity and specificity were higher than a studies conducted in Tanzania, South Gondar and Haiti where sensitivity and specificity of professionals on detection of malaria parasite were, (74.5%, 69%) in Tanzania, (78.5%, 73.7%) in South Gondar and(66.3%, 88.6%) in Haiti [22, 24, and 25].

Our finding of an overall agreement on detection of malaria parasite with reference readers was 0.67 which is defined as '*substantial*' based on the Kappa index Landis and Koch (34). The overall agreement in the current study was only slightly higher than with the study by Clendennen et al. who compared the results from 14 technologists reading of 19 malaria slides (9 positive and 10 negative) and found an inter-ratter agreement of kappa = 0.61 (37), although these was much better than with report from 4 laboratories in Mpumalanga province, South Africa, which showed a worryingly high level of disagreement (kappa=0.11%) [38]. Over all agreement in identification of different species of malaria in the current study was 0.63 which is higher than result reported in South Gondar (0.41% agreement in identification of malaria parasite with reference readers) [25].

Fourteen (19.4%) of participants reported all ten slides correctly and 79.2%, 12.5% and 13.8% of the participants reported major errors in slide 1 and slide 2 of *P.falciparum* positive slides and four negative slides respectively. Errors reported in the diagnosis of *P.falciparum* in the present study were more than those recorded in USA (3.4 % error for speciation of *P. falciparum*), Hong Kong (5% failure rate in species diagnosis of *P.falciparum)*, Canadian (22% errors in the species diagnosis of *P. falciparum*), UK (21% errors in identification of *P.falciparum*) and Democratic Republic of Congo (34.9% errors in identification of *P.falciparum)* [21, 26-27, 28, 30].

False positive results (i.e. positive when actually no malaria), were reported by 6.9% of participants. This finding is higher than 2.0% false positive result reported in Canadian study [27]. However it is lower than 7.8% false positive result reported in USA [21] and 24.6% false positive result reported in Democratic Republic of Congo [30]. This false positive result suggests that participants may often incorrectly report the presence of parasites, which could lead to unnecessary treatment or a delayed diagnosis of the true cause of illness and distract the clinician from considering other causes of fever and disease

In the current study, 55.6% and 51.4% of the participants reported minor error on each of two mixed slides(slide 3 and 4 respectively) which is much lower than the result reported in UK where proportion of minor error were 71% [28]; however, it was higher than the finding of 25% in the Peruvian Amazon [29]. Our study found out that most case of mixed malaria reported as *P. falciparum* in contrast to study conducted in Peruvian Amazon where most cases of mixed infections were reported as negative and *P.vivax* [29]. This may be due to some participants did not spent sufficient time to examine the slides and there is luck of awareness for the possibility of the presence of more than one species in blood films.

Analysis of performance of participants based on their experience in current study showed that participants who had two or more years of experience had poor agreement slides with *P.falciparum* at low parasitemia (23.2%) which is much lower than 49.2% for slides of *P.falciparum* with low parasite density reported in Peruvian Amazon [29]. These may indicate the need of refreshment training with special emphasis at low parasitemia. The challenge in detection and identification of was also reported in study conducted at Cambodia where expert level microscopists were associated with difficulty in identification malaria parasite in slide with low parasitemia [35].

8- Limitation

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

The second limitation of this study was that all participants did not count parasite density on thick blood films using parasite per microliter method which is the currently recommended technique parasitic load quantification. None of the participants reported that they correctly could count using this per microliter method of parasitic load quantification.

9- Conclusion

The overall sensitivity and specificity of participants in detection of malaria parasites were 82% and 96.5% respectively; however, they had low (74.3%) agreement in identification of different species of malaria .Especially lower agreement were reported for parasites with low density and identification of mixed infection. Participants from government health center were found to have low performances in identification of malaria parasite

10- Recommendation

- The lower agreement of the professionals with expert microscopists in identification of malaria species should be addressed by government and other quality officers, national and regional laboratories
- Providing continuous assessment and supervision
- Giving frequent in-service training to improve skills focused in identification of different species of malaria specially parasites at low density and parasite count using parasites per microliter.

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Annex 1: Questionnaire

The information was collected by principal investigator. It was collected December 1, 2013 to January 30, 2014.

Code	Question	Response Category	Remark
101	Code (FC/PC*)	_____	
102	Age in Years	_____	
103	Sex	1. Male 2. Female	
104	Where did you graduate?	1. Government 2. Private 3. Other_____	
105	What did you graduate with?	1. BSc (generic) 2. BSc (Post Basic) 3. Diploma	
106	How long has it been since you started working on malaria microscopy?	1. Less than 2 years 2. More than or equal 2 years_____	
107	How many malaria slides do you examine daily?	1. Less than 5 2. From 5-10 3. More than 10___	
108	Have you ever had an in-service training on malaria microscopy?	1. Yes 2. No	
109	If yes, how many times?	_____	
110	Is there any supervision from regional or national laboratories	1- Yes 2- No	
111	Do you use RDT for diagnosis of malaria?	1. Yes 2. No	
112	Is the microscope functional?	1. Yes 2. No	

113	What method do you use for the diagnosis of malaria	1- Thin film only 2- Thick film only 3- Thin and thick film	
114	Do you perform parasitemia count	1- Yes 2- No	
115	If yes, which methods do you use	1- +, ++, +++ 2- Parasite per micro liter/ WBC 3- Parasite per micro liter/ RBC	

Annex -2 Check list

Result reporting form for laboratory professionals

Name of health facility _____

Lab technician ☐ Lab technologist ☐

Code Lab technicians/technologist _____

Slide ID	Result				Remark
	Negative	Positive			
		Species	Stage	Parasite Load Against WBC in thick film	

Date: _____

Signature of the lab technicians/technologist_____

Annex 3: Information sheet and consent form

1- Background to the study

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

2- 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.

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

There is no risk related to participating in the study.

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

The information collected will not have any specific information including name that might break your anonymity. 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 questions, you may consult me by the following addresses:

Freshwork Ayalew (PI)
Department of Medical Laboratory Sciences, College of Health Science,
Addis Ababa University
Addis Ababa, Ethiopia.
Email: workfresh@gmail.com:
Tel: +251912009862

Signature: _____

Date: _____

5- Consent form

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

Signature of participant: _____

Annex 4- consent form for blood donors

Purpose

My name is freshwork ayalew and I want to develop a collection of malaria slides for the purpose of evaluating the performance of laboratory technicians/ technologists in the diagnosis of malaria. So I request to collect a blood samples to make large number of identical blood films (smears).

Procedures

I will have a 3 ml.–5 ml. sample of blood (1/2 teaspoon) extracted for this study. The blood will be assessed by microscopy to check the type of malaria present, and the number of parasites. If found suitable, it will be placed on slides and used for evaluating the performance of laboratory technicians/ technologist working in eleven health facilities of Hawassa city.

Risks and discomfort

I understand that the risks involved in this study are minimal. They include the discomfort of drawing a sample of blood, rare bruising and infection at the site of needle stick, and very rarely, fainting. New needles will be used for each patient so there is no risk for transmitting diseases.

Confidentiality

All my identifiable records and information will be kept strictly confidential, and remain in secure storage with the principal investigator collecting the blood sample.

Contact information

For further inquiries, I can contact the following persons:

Freshwork Ayalew (PI)

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