ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Performance Evaluation of Laboratory Professionals on Manual assessment of Peripheral blood cells morphology and associated factors in government hospital laboratories of Addis Ababa, Ethiopia

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A research thesis submitted to the Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Clinical Laboratory Management and Quality assurance)

June, 2018

Addis Ababa

ADDIS ABABA UNIVERSITY

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This is to certify that the thesis prepared by Yeshi Yimer Abebe, entitled:

Performance Evaluation of Laboratory Professionals on Manual assessment of Peripheral blood cells morphology and associated factors in government hospital laboratories of Addis Ababa, and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Laboratory Management and Quality assurance) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Acknowledgment

First of all, I would like to thank the almighty God who gave me the courage and power to finish this thesis.

Secondly, it's my pleasure to acknowledge Tirunesh Beijing general hospital and Addis Ababa University, School of Allied Health Science and Department of Medical Laboratory Sciences for providing me this opportunity and for the financial support that I received.

I am most grateful to my advisors Fatuma Hassen and Dr. Aster Tsegaye for dedicating their time in reading and correcting my thesis.

I want to express my great thanks to the other two pathologist advisors: D/r Mesfin Asefa and D/r Yonas Bekuretsion for preparing and validating the proficiency testing slides and for ideal support

I would also like to extend my thanks to Mr. Fanuell Zewdu for preparing and validating RBC inclusion (malaria) panel slide and to the study participants working in the 13 government hospital laboratories of Addis Ababa for their willingness to participate.

Finally my deep appreciation goes to my colleagues who were positive throughout the course year and research paper.

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Abbreviations

ALL	Acute lymphoid leukemia
AML	Acute myeloid leukemia
AOR	Adjusted odds ratio
APL	Acute promylocytic leukemia
CLL	Chronic lymphoid leukemia
CML	Chronic myeloid leukemia
EDTA	Ethylene diamine tetra acetic acid
EQA	External quality assessment
MAPBCM	Manual assessment of Peripheral blood cells morphology
PI	principal investigator
RBC	Red blood cell
SOP	Standard operating procedure
TASH	Tikur Anbessa Specialized Hospital
UK	United Kingdom
WBC	White blood cell

Abstract

Background: Microscopic examination of Wright's stained blood film by skilled professionals has remained the standard laboratory method for assessment of peripheral blood cells morphology. However, assessment of blood cells morphology with this method is problematic since interpretation of result need considerable experts.

Objective: The objective of the study was to evaluate the performance of laboratory professionals on manual assessment of Peripheral blood cells morphology (MAPBCM) and associated factors in government hospital laboratories of Addis Ababa.

Methods: A cross sectional study was conducted from April 1 to May15, 2018. A total of 202 medical laboratory professionals were participated with a response rate of 90.6%. Self-administered questionnaires and observational checklist were employed to assess factors and routine activity. Ten panel slides, eight from cases with hematological abnormality, one from healthy person and one RBC inclusion (malaria) were used to evaluate laboratory professionals' performance in MAPBCM. Data were entered and analyzed using SPSS version20 and p-value <0.05 was taken as statistically significant.

Results: More than half of participants (56.9%) were male and the mean age of the participants was 30 (SD=5.1) years. All participants had never taken training, 158(78.2%) of them had sometimes done smear review for flagged automated result. Among13 hospitals 11(84.6%) had daily microscope preventive maintenance. Of the participants, 31 (15.3%), 32(15.8%), 23(11.4%) correctly reported all red blood cells (RBC), white blood cells (WBC) and platelets morphology panel slides individually. performance of professionals in assessment of WBC (p=0.028), and platelet (p=0.005) had statistical significant association with experience in hematology laboratory. The overall agreement between reference and participants reading was 55%, 52%, 41%, 35% for RBC, WBC, platelet and malaria detection, respectively.

Conclusion: Overall low agreements were found between reference reading and participants reading in MAPBCM. Performance level of laboratory professionals on assessment of WBC morphology and platelets estimation was related to experience in hematology laboratory. Thus, more practical focused training with supervision is recommended.

Key words: peripheral blood smear, blood cell morphology, laboratory professionals

1. Introduction

1.1 Background

Peripheral blood cells include red blood cells (RBC), white blood cells (WBC) and platelets. Morphology of these cells is assessed from optimal area of properly prepared and stained peripheral blood smear by microscope. Before assessing cell morphology and categorizing as normal and abnormal, there should be knowledge of normal cell morphology. Normal RBCs is biconcave disk shape without nucleus and organelle, 7-8 μm in diameter and 90 fl of average volume. It has approximately 2-3 μm diameters of central pallor area and reddish-orange appearance with Romanosky stain. Deviation in shape, size, and distribution, concentration of hemoglobin, color and appearance of the cells and presence of inclusions thought as abnormal and may diagnose various forms of anemia (1).

Diagnostic abnormal RBC morphology includes; variation in shape (Elliptocytes, Blister, Acanthocytes, Schistocytes, Spherocytes, Keratocytes,), variation in red cell distribution (agglutination and Rouleaux formation), variation in size (Macrocytes and Microcytes), variation in hemoglobin concentration (Hypochromia and Polychromasia) and also Howell-Jolly bodies, papenhemer bodies, basophilic stippling and henz bodies are listed as inclusion(1, 2).

Normal WBC includes: Neutrophils, eosinophils, basophiles, lymphocytes and monocytes. Mature neutrophil measures 12–15μm in diameter, acidophilic cytoplasm with fine granule, 2-5 lobed nucleus and constitute 40-75 % of entire leucocyte. Neutrophilia may associate with chronic myelogenous leukemia. Eosinophil is slightly larger than neutrophile with 2-3 lobed nucleus, large reddish orange granules, basophilic cytoplasm, accounts 1-6% of total leukocyte and marked eosinophilia related to eosinophilic leukemia. Basophil accounts <1% from total leukocyte, has diameter of 10-14μm, nucleus covered by purple-black granules. Basophilia may relate with chronic myeloid leukemia. Lymphocytes account 20-45% of total leukocytes, 10-16μm in diameter, weakly basophilic cytoplasm. Absolute lymphocytosis may associate with acute and chronic lymphocytic leukemia. Monocyte is 12-20μm in diameter with fine granules in opaque greyish-blue cytoplasm and an irregular often lobulated nucleus and measure 2-10% of entire leukocytes. Monocytosis is seen in acute and chronic myeloid leukemia (3, 4).

Platelets (thrombocytes) are nucleated disc type cells, are approximately 1 to 4 micrometer diameter and have reddish-purple granules over pale blue cytoplasm. Normally platelets account approximately 150,000 to 350,000 cells/ microliter of blood and it may arise due to malignancy (5).

In hematologic diseases, young, immature, reactive and atypical forms of leukocytes, like blasts (monoblasts, lymphoblasts, erythroblasts, myeloblasts) can be identified in peripheral blood. The detection of blast cells in the peripheral blood is one of the signs that determine the presence of acute leukemia. Since acute leukemia does not have disease specific manifestation in initial stage and its manifestation is largely similar to anemia. This situation may be clarified by clinical analysis of blood. Abnormal leukocyte count with detection of blast cells in the peripheral blood is correlated with acute leukemia (6).

Anemia and leukemia are worldwide problem. Globally, 1.62 billion people (95% CI: 1.50–1.74 billion) which is 24.8% of the population are affected by anemia. Developing nations like Africa has highest burden of anemia, especially women of child bearing age and children (7). In those developing nations where advanced investigations may not be readily available, peripheral blood morphology examined by skilled professionals from a well prepared well stained blood smear is important for evaluation of anemia patient for care as well as monitor treatment outcomes. It is also rapid and cheaper for platelets estimation (8, 9).

Now a days even if, the proportion of blood-count samples that require a blood smear has steadily diminished due to development of sophisticated automated blood cell analyzers, blood smear remains a crucial diagnostic aid, it may be requested by physicians or initiated by laboratory staff, and cannot be totally replaced by automated analyzer but they are complementary to each other, both methods provide complete important information on about kind and number of cells which indicate important medical disorder (10, 11, 12). 80% of critical decision-making inpatient care is influenced by laboratory testing. Hence, quality in laboratory has huge impact on diagnosis and patient management as about this percent of all diagnosis is made on the basis of laboratory tests (13, 14).

1.2 Statement of the problem

Developing quality culture in the laboratory through quality laboratory management system is used to deliver timely, precise and accurate results and to meet patients need as well as satisfaction (15). A study done in United States of America (USA) indicated that 6% to 12% of laboratory errors guide the patients at risk of inappropriate care and potentially of adverse events, and from 26% to 30% of errors have a negative impact on other aspects of patient care (16). Those errors may arise due to laboratory professionals incompetency so assessing laboratory personnel competency and improving the gaps accordingly is required (17).

Morphological assessment of blood cells using the microscope is one of important laboratory test and used to verifying result generated by automated analyzer as quality control tool. It helps to recognize and identify clinically significant abnormalities which cannot be detected and identified or flagged by analyzers. These include elliptocytes, target cells, sickle cells, acanthocytes, teardrop cells, nucleated/immature red cell, rouleaux, Howell Jolly bodies, toxic granulation, blasts and abnormal platelet granulation (18). In the college of American pathologist (CAP) Q Probes program study with 263 participating hospitals and independent laboratories, the rates of manual smear review varied among participants, with a median of 26.70% (19).

Manual assessment of blood cells using microscope has been set as the gold standard method (20, 21, 22). However, it is subject to individual interpretation error of images and correctness of result interpretation as it depends on the quality of the smear, the quality of the stain, the quality of the microscope and the skill, experience and training status of the microscopist (23). For instance, study from United Kingdom (UK) shows over all low performance of participant professionals and relatively higher rate of diagnosis on prioritization of abnormal cell morphology and trend to report more relevant features for diagnosis in more experienced professionals than less experienced professionals (24).

In Ethiopia, study conducted on utilization of external quality assessment in case of proficiency test at government hospitals of Addis Ababa. Proficiency test result on peripheral blood morphology using digital images revealed 56.2% external quality assessment failure rate (25). However, the performance of laboratory professionals on manual assessment of peripheral blood cells morphology (MAPBCM) and associated factors are not known.

Hence, this study aimed to evaluate performance of laboratory professionals on MAPBCM and associated factors. The study will point out the problems in this regard and it gives clear information of the gaps for further quality improvement on MAPBCM.

1.3 Significance of the study

Evaluating performance of laboratory professionals on MAPBCM and assessing factors that cause performance difference between professionals helps to identify area of improvement to provide high quality service on MAPBCM. In practice, MAPBCM depends on smearing and staining quality of smear, quality of microscope and skill as well as experience of professionals. There is no supervision and training to scale up professional performance on MAPBCM in our country. So this study is a good indicator to identify any gaps or source of error and the need for improvement of the gap analyzed.

Therefore, current study, on performance evaluation of laboratory professionals on MAPBCM and associated factors which will be an important source of information for the stakeholders, hospital administrators and quality officers in order to plan for overcoming gaps and implement continuous supervision for improvement. This study has also advantage for patients in long run after identifying gaps and implementing quality improvement strategies by stake holders, there will be improvement in diagnosis and treatment of diseases for which peripheral blood morphology is indicated.

2. Literature Review

A microscopic examination of well-prepared and well-stained blood smear by a skilled laboratory professional is clinically useful in a number of disease conditions. Especially in resource limited settings where advanced technologies may not be readily available, peripheral blood morphology is important for evaluation of anemia and leukemia as well as detect abnormalities on WBC and platelets both quantitatively and qualitatively (8, 9, 26). Though given little attention, blood smear remains a crucial diagnostic aid and cannot be totally replaced by automated analyzer rather both methods are complementary to each other (10, 11, 12). The paucity of published researches regarding performance of medical laboratory professionals on manual peripheral blood morphology and factors affecting them implies the lack of attention especially in the developing world where exploiting its clinical utility is important until more advanced technologies are widely accessible.

2.1 Performance of laboratory professionals on MAPBCM

A study conducted in hospitals of New Orleans city in the state of Louisiana to assess the competency of 75 technologists as well as to construct valid competency measurement tool through comparing of color transparency and slide challenge methods. They distributed slide of five peripheral blood morphology containing Lymphoblast, Reactive lymphocytes, Band neutrophils (myeloid precursors), Monocytosis and from healthy person. The study revealed that of the participants, 23% of technologist failed to recognize the small lymphoblasts as abnormal cells, 5% failed to recognize myeloid precursors and 3% not identified normal WBC correctly (27).

In another study conducted in United Kingdom (UK) on analysis of proficiency testing result huge skill gap in identifying PT samples was noted. The finding showed that only 23 % (176/772) of participants were able to report lymphoid malignancy correctly and correct report of oxidative hemolysis was done by 46% (358/772) of participants. Only 13 % (104/772) of participants were able to report correct result for both lymphoid malignancy and oxidative hemolysis (24).

A cross-sectional study was conducted in Tigray, northern Ethiopia to assess the performance of 46 laboratory professionals in microscopic diagnosis of malaria, by using six panel slides including positive and negative. The overall agreement between the study participants and the reference reader in malaria detection was 79% (kappa = 0.62) and Participating in refresher training on malaria microscopy (Adjusted Odds Ratio (AOR = 7, CI = 1.5–36.3)) and malaria epidemic investigation (AOR = 4.1 CI = 1.1–14.5) had statistical significant association with detection rate of malaria parasites (28).

Another study conducted in defense health facilities in Addis Ababa and its surrounding areas, Ethiopia, to evaluate performance of 60 malaria microscopists by using pre-validated panel slides. Result indicated Overall, fair agreement (71.4%; Kappa: 0.4) in detection of malaria parasite between the study participants and expert readers (29).

As far as my literature search goes, there is no published study from Africa regarding MAPBCM performance of medical laboratory professionals though one study from Ethiopia identifies 56.2% proficiency testing failure rate of government hospital laboratories on assessment of peripheral blood cells morphology based on digital images (25).

2.2 Factors affecting the performance of professionals on MAPBCM.

Study conducted in Walden University aimed to correlate the performance accuracy of Peripheral blood differentials with educational level. A total of 10 peripheral blood smears were given to clinical laboratory technician and clinical laboratory scientists to perform differential. Result indicates that significant discrepancy in the levels of accuracy between clinical laboratory scientists and clinical laboratory technicians. Results of peripheral blood differentials performed by clinical laboratory technicians were relatively questionable (30).

Another study conducted in UK on analyzing the responses of participants in proficiency testing to identify successful strategies as well as sources of error. The findings indicated that biomedical scientists who regularly issued morphological reports showed a trend to report features more relevant to diagnosis than those who did not for both red cells and white cells. Effective prioritization of abnormal cell forms for both white and red cell features was also demonstrated among biomedical scientists who regularly issued morphological reports. This

indicates biomedical scientists who regularly issued morphological reports had a higher overall rate of better diagnosis (24).

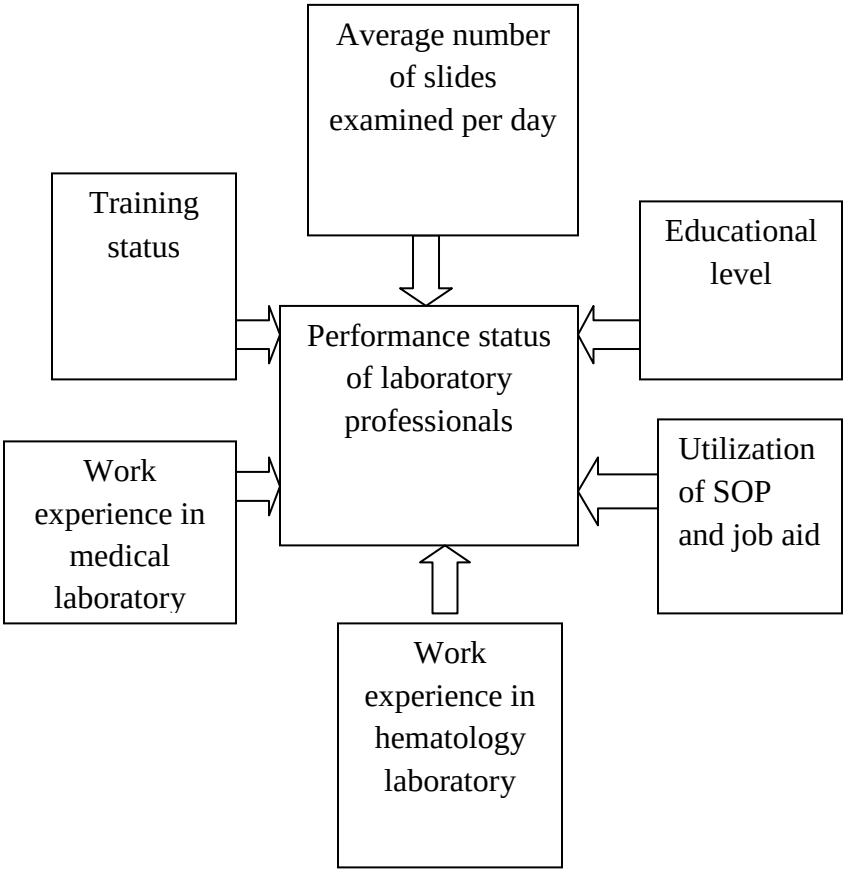

Figure 1; Conceptual framework

3. Objectives

3.1. General objective

To evaluate the performance of laboratory professionals on manual assessment of peripheral blood cells morphology (MAPBCM) and associated factors in government hospital laboratories of Addis Ababa.

3.2. Specific objectives

1. To determine the performance of laboratory professionals on MAPBCM.
2. To identify factors associated with the performance of professionals on MAPBCM.

4. Materials and methods

4.1. Study area

The study was conducted in Addis Ababa, capital city of Ethiopia, which is located at the heart of the country, and covers a Landmass of 540 square kilo meters. The city includes three layer of administrative government which is city government at the top, 10 sub cites in the middle and 116 woreda with total population of 2,739,551 (31). There are 46 hospital laboratories of which, 5 are federal, 6 regional, 1 defense, 1 prison and 1police, 30 private and 2 NGO-supported. In addition, there are 70 public and 4 NGO-supported health centers laboratories, and 7 public, 500 private and 31 NGO supported clinics laboratories (32). From 14 government hospitals, federal police hospital was excluded from the study for pre testing of questioner. Therefore, the study was conducted on laboratory professionals working in 13 government hospitals (TASH, St Paul's hospital millennium medical college, St peters specialized hospital, Amanuel mental specialized hospital, ALEART (All African leprosy ,tuberculosis rehabilitation training center), Ras Desta Damtaw memorial hospital, Yekatit 12 hospital medical college, Dagmawi minillik memorial hospital, Gandi memorial hospital, Zewditu memorial hospital, Tirunesh Beijing general hospital, Federal armed force hospital, Federal prison hospital) laboratories to address professionals who are working in different condition and to get more representative data. Since, hospital laboratories are highly exposed to manual peripheral blood morphology service due to complication of cases what they manage, and government facilities are easily accessible than non-government facilities, priority is given to government hospital laboratories.

4.2. Study design and period

Cross sectional study was conducted from April 1 to May 15, 2018 by distributing a total of 10 panel slides.

4.3. Population

4.3.1. Source population

All laboratory professionals who were working at government hospital laboratories of Addis Ababa

4.3.2. Study Population

Those laboratory professionals who were active on duty during the study period, who fulfill inclusion criteria and working at 13 government hospital laboratories' of Addis Ababa.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

Those professionals who had greater than six month of experience were included in the study.

4.4.2 Exclusion criteria

Those professionals who were permanently assigned other than hematology workstation were excluded

4.5. Study variables

4.5.1. Dependent variables

- Performance level of laboratory professionals

4.5.2. Independent variables

- Age
- Utilization of SOP and job aid
- Average Number of slides examined per day
- Educational level
- Training status
- Work experience
- Work experience in hematology laboratory

4.6. Sample size calculation and Sampling method

4.6.1. Sample size calculation

Sample size for study participant was determined by single population proportion formula. Because of the absence of previous study, p=50 %, q=1-p (0.5) were taken, With Margin of error (d) = 5%, Z-score at 95% confidence interval = 1.96, the formula for calculating the sample size (n) is

$n=z^2pq/d^2$

$n= (1.96)^2(0.5)(0.5)/(0.05)^2$

=385

Since a total of 422 professionals in 13 government hospital laboratory which is below 10,000.So correction factor was applied based on the finite population formula (nf). Therefore, the sample size were reduced to; nf = n/1+n/N

nf=202

Considering 10% non-response rate, the sample size becomes 202 +21 = 223

4.6.2. Sampling method

Study participants who were working in hematology workstation through rotation were selected by simple random sampling after allocating sample size proportionally. However, those who were working in hematology workstation without rotation for greater than six months were all included.

4.7. Measurement and Data collection

4.7.1. Data collection procedure

Data were collected by distributing a total of ten panel slide of peripheral blood smear. Eight slides from cases with hematological abnormality and one slide from healthy person were used to assess the ability of professionals in identifying abnormal and normal cell morphology of WBC, RBC and platelet. Additionally, one malaria slide was used as RBC inclusion and to assess the consciousness of participants towards malaria when they observe cell morphology. Moreover, factors related to the performance level of professionals on MAPBCM were assessed using self-administered questionnaires and availability of sufficient reagents, sops and job aid, daily microscope preventive maintenance, were assessed by observational checklist. Study participants were oriented primarily for requirements that need to be recognized. Time required to review peripheral blood smear is an average of 4.2 minute (22). Each participant was requested to examine coded slides and they were reported each slide with in an average of approximately 5 minutes. Data collector/PI also collected captured information from the panel slides as well as the questionnaires and retrieved the panel slides after each participant completed the test.

4.7.1.1 Panel slide preparation

Four milliliter of whole blood was collected from patients that attended in Tikur Anbessa Specialized Hospital (TASH) pathology department with cases of hematologic abnormalities of WBC, RBC, and platelet and from healthy person. Blood was collected after obtained informed consent and the blood samples were transferred in to separate EDTA containing glass tubes and processed within one hour of collection to avoid morphological changes in blood cells (33). Then, a drop of well-mixed, EDTA anti coagulated blood was placed at one end of a clean dry slide and spread using a second spreader slide at 25–30° and leaving an oval feathered edge. The slides were air dried and labeled with dimoned marker before stained by wright stains (34). As the same time complete blood count (CBC) was performed for each sample with sysmex 2000i hematology analyzer in TASH laboratory hematology department.

4.7.1.2 Panel slide characteristics

Slides were prepared from most frequently occurring cases of the country with hematologic abnormalities of RBC, WBC and platelet and from healthy person. The two pathologists interpreted the blood smears individually and agreed with the result, complete blood count were also done with Sysmex 2000i hematology analyzer. RBC inclusion (malaria) slide was interpreted as malaria of plasmodium falciparum (++). All ten panel slides include:

1. Thrombocytosis with hyper segmented neutrophil, platelets count of 1,000,000 cells /microliter of blood.
2. Acute myeloid leukemia (AML) of myeloid blast with total WBC count of $133.56x10^3$ cells /microliter of blood.
3. Chronic lymphoid leukemia (CLL) with lymphocytosis, total WBC count of $339.34x10^3$cells /microliter of blood and 95.4% of lymphocyte differential.
4. RBC inclusion(malaria) of plasmodium falciparum(++)
5. Acute promylocytic lukemia (APL) of promylocyte with total WBC count $35x10^3$ cells /microliter of blood
6. Anisocytosis, pokilocytosis other than tear drop cells and tear drop cells with low hemoglobin count ,low (mean cell volume(MCV),mean cell hemoglobin(MCH) and mean cell hemoglobin concentration (MCHC)), high red cell diame width (RDW).
7. Acute lymphocytic leukemia (ALL) with lymphoblast, total WBC counts 23.1 $x10^3$ cells /microliter of blood with 86% lymphocyte differential count.
8. Chronic myeloid leukemia (CML) with myloblast ,promylocyte, metamylocyte, mylocyte, total leukocyte count 362.2 $x10^3$ cells /microliter of blood ,81.3% neutrophil differential
9. Pancytopenia ,2.7 $x10^3$ cells /microliter of blood ,platelet count was below detectable limit of analyzer(undetectable)
10. Health person with normal WBC ,RBC and Platelet count

4.7.1.3 Administration of the Slide Panel Test

After arranging slides in to ten sets including malaria slide, distribute to participant hospitals with log sheet for result recording. Five minutes per slide were allocated for every participant equally to evaluate three cell classes and considered longer time when they observed malaria

slides confidentially without disclosing it for them. 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.

4.7.1.4 Questionnaire

A self-administered questionnaire including information on the socio-demographic characteristics, educational background, work experience as medical laboratory professionals as well as experience in hematology laboratory, average number of slides examined per day, training status, utilization of standard operation procedure (SOP)and job aid, question related to experience on measure for flagged automated result and trend of referral on difficulty of routine work were included and distributed to participant professionals.

4.7.1.5 Observational checklist

Observational checklist includes information on availability of sufficient reagents, SOP, job aid, daily microscope preventive maintenance check and peripheral blood morphology report by the laboratory.

4.8. Data Quality Assurance

Pre analytical phase; quality of data was assured in this phase by isolating the real cases through those pathologist and involving them in preparation, staining as well as interpretation of panel slides. Follow SOP for sample collection, that is, site selection, without mixing with tissue fluid, optimal volume of blood with considerable volume of anticoagulant including best anticoagulant choice for cell morphology. SOP also used when smearing and staining of panel slides (filtration of stain, keeping body and feather of thin blood film, properly drying and staining with appropriate reagent and time). As quality control, appearances of mature blood cells were checked by staining slides from normal blood with panel slides. One hemato-pathologist from St. Paul hospital millennium medical collage pathology department and one pathologist from Tikur Anbessa Specialized hospital (TASH) pathology department were involved in the isolation of cases, preparation and validation of peripheral blood morphology panels and only those slides whose reading was agreed by both comparable with analyzer results were used as panel slide to minimize inter observer error. RBC inclusion (malaria) was validated by expert microscopist who is pre-qualified and certified by WHO. Data quality was also assured

by pretesting of the questionnaire on 5% of the participating professionals in police hospital and corrected it as needed.

Analytical phase; during this phase there were intensive supervision by data collector/ PI. Time needed to report each slide was optimized and each participant was given equal amount of time. Participants from same facility were read the slides using same microscope. The quality of the microscopes was checked by the PI (laboratory technologist who had six years' experience on the field) using the quality control slides through checking of mature cells for color of nucleus, cytoplasm and granules by using checklist (35).

Post analytical phase; after participants were fill necessary information data collector/ PI was retrieved slides and collected captured information confidentially.

4.9. Data analysis and interpretation

Data were entered, cleaned and analyzed by using SPSS windows version 20. Association between levels of professionals performance in assessment of WBC morphology, RBC morphology and platelet estimation were compared with independent variables that were collected by 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), multiple logistic regressions for those variable of p value in chi-square <0.2 was computed to observe significance of association, percent agreement and kappa score also were calculated to assess the agreements of participants with reference readers on MAPBCM. The cut off proposed by Landis and Koch as standards for strength of agreement of Kappa coefficient, as adopted by others, were used in this study: < 0.00 = Poor (less than chance agreement, 0.00-0.20 = Slight; 0.21-0.40 = Fair; 0.41-0.60 = moderate; 0.61-0.80 = Substantial, 0.81-1.00 =Almost Perfect agreement (36, 37). Results were presented in tables and figures.

4.10. Ethical considerations

This study was approved by department research and ethics review committee (DRERC) of the Addis Ababa University, Department of Medical Laboratory Science. Ethical clearance was given from Addis Ababa health bureau and from each federal hospitals ethical committee, official letters was written to the participating hospitals. Consent forms were prepared in English to be read and sign (if agree) 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. The poor performance of participants will be noticed to stockholders and managed accordingly.

4.11. Dissemination of the result

The findings of this study will be presented to department of medical laboratory science and will be disseminated to Addis Ababa health bureau and participating federal hospitals. The results of this study will also be disseminated to the scientific community through scientific presentation and publishing in peer reviewed journals.

4.12. Operational definitions

External quality assessment failure rate is total number of challenges graded as an acceptable for a single test parameter divided by total number of challenge distributed times hundred.

Healthy person; individual without any sign and symptoms of disease that affect hematologic parameter

Permanent bases; refers to assigning of laboratory professionals in one workstation more than six month

Rotation bases; refers to assigning of laboratory professionals in one workstation six month and below.

5. Results

5.1 Socio demographic characteristics of study participants

In this study a total of 223 laboratory professionals were approached. Out of these 202 study participants were appropriately respond all questions, which make the response rate 90.6%. Of these, 105(52%) were generic BSc holders, followed by advance standing BSc 58(28.7%) and more than half of participants 115 (56.9%) were male. The mean age of the participants was 30 (SD= 5.1) years. Among the participants,70(34.7%) had four and less years of experience,88(43.6%) of participants had experience between five to eight and the rest had more than nine years' experience in medical laboratory. Similarly,73(36.1%) had three and less years of experience,51(25.2%) of participants had experience between four to six and the rest had more than seven years' experience in hematology laboratory and all participants had never participated in training about manual assessment of peripheral blood cell morphology. **(Table 1).**

Table 1-Socio demographic characteristics of participants of performance evaluation of peripheral blood cells morphology assessment in thirteen government hospitals of Addis Ababa, Ethiopia, 2018

Variables	Number	Percent
Age		
22-26	51	25.2
27-31	91	45.1
32-36	42	20.8
>37	18	8.9
Sex		
Male	115	56.9
Female	87	43.1
Educational status		
Diploma	31	15.3
BSC(generic)	105	52
BSC(post basic)	58	28.7
Masters	8	4
Experience in medical laboratory		
< 4 years	70	34.7
5-8 years	88	43.6
9-12 years	23	11.3
>13	21	10.4
Experience in hematology laboratory		
<3 years	73	36.1
4-6 years	51	25.2
7-9 years	42	20.9
>10 years	36	17.8
Participation in training		
Yes	0	0
No	202	100

5.2 Assessment of participant hospitals related to peripheral blood cells morphology service

Based on the result of observation checklist, among13 participant hospitals, 10 (76.9%), 9(69%), had SOP and job aid for peripheral blood morphology, respectively. On the other hand there was no peripheral morphology reported by 5(38.4%) of laboratories during assessment period. Similarly, 11 (84.6%) of hospitals had daily microscope preventive maintenance and 12 (92.3%) of laboratories had sufficient reagent for peripheral blood morphology; however, professionals who are working in some hospitals said that even those reagents were sufficient, quality of it were debatable**. (Table 2)**

Table 2-Assessment of peripheral blood cells morphology service in thirteen government hospitals of Addis Ababa, Ethiopia, 2018

Variables	Number	%
Availability of SOP		
Yes	10	76.9
No	3	23.1
Availability of job aid		
Yes	9	69
No	4	31
Peripheral blood morphology report by laboratory		
Yes	8	61.6
No	5	38.4
Microscope daily preventive maintenance		
Yes	11	84.6
No	2	15.4
Availability of sufficient reagent		
Yes	12	92.3
No	1	7.7

5.3 Routine practice of participant on manual assessment of peripheral blood cells morphology

Participants were also asked about their routine practice for peripheral blood morphology. Among participants, 146(72.3%) of the participants stated that they examined less than five peripheral blood morphology slides per day and as 182(90.1%) of participants report, laboratories were accepted five and less blood morphology request per day. Considerable number of participants 168(83.2%) utilize sop and job aid during peripheral blood cell morphology evaluation. On the other hand, 158(78.2%) of participant responded that they had experience of peripheral blood smear review for flagged automated result occasionally. Similarly, only 43(21.3%) of participant responded as there were trend to report pathologist/hematologist if there were any difficulties on peripheral blood cell morphology assessment during their daily practice. **(Table 3)**

Table 3-Routine practice of participants in manual assessment of peripheral blood cells morphology in thirteen government hospitals of Addis Ababa, Ethiopia, 2018

Variables	Number	Percent
Number of slides examined per day		
<5	146	72.3
From 5-10	31	15.3
>10	1	0.5
None	24	11.9
Utilization of sop and job aid		
Yes	168	83.2
No	34	16.8
Number of blood morphology request received by the laboratory per day		
Less than five	182	90.1
Greater than six	20	9.9
Practice of peripheral blood smear review for flagged automated result		
Always	34	16.8
Sometimes	158	78.2
Never	10	5
Measure for difficulties on identification of cell morphology		
Refer to pathologist	43	21.3
Refer on atlas	95	47
Communicate with other staff	64	31.7

5.4 Performances of laboratory professionals on manual assessment of peripheral blood cells morphology

Of 202 participants, 121(59.9%) were unable to recognize hyper segmented neutrophil. Blood smear corresponding to AML, ALL and CML cases majority recognized blast cells 137(67.8%) from AML slide, 84(41.6%) from ALL, and 140(69.3%) of participants from CML slide. Among those who were able to recognized blast cells, only 46 or (22.8%) of total participants were identified myeloblast correctly from AML case and considerable number, 136(67.3%) of participants were failed to report myeloblast from CML case. Similarly, 179(88.6%) of participant were failed to reported lymphoblast from ALL case. CLL was described as Lymphocytosis (small lymphocyte) by reference readers, 150(74.3%) of participants were failed to report it. Promyelocyte was correctly reported by 28(13.9%) of participants from APL cases and 44(21.8%) of participant from CML cases including myelocyte and metamyelocyte.

Participant reports were observed for WBC estimate through case of CML with marked cytosis, 189 (93.6%) of participant were recognized it correctly. On the other hand, participant reports were also analyzed corresponding to RBC morphology. Among the participants, 134(66.3%) were reported anisocytosis correctly and pokilocytosis other than tear drop cells was identified by 71(35.1%) of them. Participant reports were analyzed for tear drop cells independently besides, it showed 31(15.3%) of participants could recognize it. Malaria of *Plasmodium falciparum* (++) was included as RBC inclusion, only 70(34.7%) of participants were able to detected and 58(28.7%) of them could recognize including of species as *Plasmodium falciparum*. In regard to platelet estimate, 111(55%) and 89(44.1%) of participants were failed to report thrombocytosis and thrombocytopenia, respectively. Slide from healthy person was also assessed for RBC, WBC and platelet. Of the participants, 113(55.9%) were reported WBC as normal in count as well as maturation, 126(62.4%) were responded as normocytic normochromic RBC without any shape variation and only 45 (22.3%) were reported as normal platelet estimate from slide of healthy person. **(Table 4)**

Table 4- Performance of laboratory professionals on manual assessment of peripheral blood cells morphology in thirteen government hospitals of Addis Ababa, Ethiopia, 2018

Slide number	Diagnosis	Case identified		Yes		No	
				Number	%	Number	%
001	Thrombocytosis	Thrombocytosis		91	45	111	55
		Hyper segmented neutrophil		81	40.1	121	59.9
002	AML	Blast		137	67.8	65	32.2
		Myeloblast		46	22.8	156	77.2
003	CLL	Lymphocytosis		52	25.7	150	74.3
004	Parasitic infection	RBC inclusion (malaria)		70	34.7	132	65.3
		P falciparum		58	28.7	144	71.3
005	APL	Promyelocyte		28	13.9	174	86.1
006	Megaloblastic disease	Anisocytosis		134	66.3	68	33.7
		Pokilocytosis other than Tear drop		71	35.1	131	64.9
		Tear drop cells		31	15.3	171	84.7
007	ALL	Blast		84	41.6	118	58.4
		Lymphoblast		23	11.4	179	88.6
008	CML	Leukocytosis(marked)		189	93.6	13	6.4
		Blast		140	69.3	62	30.7
		Myeoblast		66	32.7	136	67.3
		(Promyelocyte, mylocyte and metamyelocyte		44	21.8	158	78.2
009	Pancytopenia	Leukopenia		124	61.4	78	38.6
		Thrombocytopenia		113	55.9	89	44.1
010	Healthy	Normal	WBC	113	55.9	89	44.1
			RBC	126	62.4	76	37.6
			Platelets	45	22.3	157	77.7

5.5 performances status of laboratory professionals in terms of three cell types

Among 202 participants**,** 31(15.3%) correctly reported all RBC morphology, 32(15.8%) correctly identified all major WBC morphology and 23(11.4%) correctly estimate platelets from all distributed proficiency testing panels of RBC, WBC and platelets respectively. **(Table 5).**

Table 5-performances of laboratory professionals in terms of three cell types

Variables	Number	Percent
All RBC slides correctly reported	31	15.3
Not all RBC slides correctly reported	171	84.7
Major WBC slides correctly reported	32	15.8
Not all major WBC slides correctly reported	170	84.2
All platelet estimation slides correctly reported	23	11.4
Not all platelets estimation slides correctly reported	179	88.6

***NB**: RBC morphology panel slides include; Slide 6 (anisocytosis), Slide 6 (pokilocytosis) and Slide 10 (normocytic normochromic).*

Major WBC panel slides include; slide1 (hyper-segmented neutrophils), slide number 8(leukocytosis with blast cells), slide 9(leukopenia) and slide10 (mature WBC with normal count).

Platelets estimation slides include; Slide1 (thrombocytosis), Slide 9 (thrombocytopenia) and Slide 10(normal platelet count)

5.6 Performance of laboratory professionals on MAPBCM and associated factors

There was association between numbers of slides examined per day with performance of laboratory professionals on assessment of RBC morphology (p=0.036) and WBC morphology (<0.001). Experience in hematology laboratory had an association with performance of professionals on assessment of WBC morphology (p=0.002), platelet estimation (p<0.001). Performance of professional on assessment of WBC morphology had also an association with experience in medical laboratory (p=0.005). Association between training status and performance level of participants was not computed because no one had training. **(Table 6)**

Table 6- Performance of laboratory professionals on MAPBCM and associated factors

Variables	**All RBC morphology panel slides correctly reported**			**All major WBC morphology panel slides correctly reported**			**All Platelets estimation panel slides correctly reported**		
	Yes	**No**	**p-value**	**Yes**	**No**	**p-value**	**Yes**	**No**	**p-value**
Age									
22-26	6	45	0.374	8	43	0.188	5	46	0.98
27-31	15	76		13	78		11	80	
32-36	5	37		5	37		5	37	
>37	5	13		6	12		2	16	
Educational status									
Diploma	4	27	0.833	3	28	0.606	0	31	0.057
BSC(generic)	15	90		16	89		11	94	
BSC(post basic)	11	47		11	47		10	48	
Masters	1	7		2	6		2	6	
Experience in medical laboratory									
<4 years	6	64	0.109	14	56	0.005	6	64	0.169
5-8 years	14	74		7	81		8	80	

9-12 years	5	18		3	20		4	19	
>13	6	15		8	13		5	16	
Experience in hematology laboratory									
<3 years	8	65	0.252	15	58	0.002	4	69	<0.001
4-6 years	6	45		1	50		0	51	
7-9 years	9	33		5	37		9	33	
>10 years	8	28		11	25		10	26	
Participation in training									
Yes									
No	31	171		32	170		23	179	
Number of slides examined per day									
<5	19	127	0.036	14	132	<0.001	1	23	0.341
From 5-10	10	21		17	14		16	130	
>10	0	1		0	1		6	25	
None	2	22		1	23		0	1	
Utilization of sop and job aid									
Yes	26	142	0.91	30	138	0.081	19	149	0.939
No	5	29		2	32		4	30	

5.6.1 Multiple logistic regressions for performance of participants in assessment of RBC morphology with associated factors

Multiple logistic regressions was calculated for variables with p value for chi squire was ≤0.2 and no Statistical significant association was observed between experience in medical laboratory and performance of laboratory professionals on assessment of RBC morphology. However, those who examine 5-10 slides per day are 2.74 times more likely to perform well than those who did not perform per day , (AOR 2.74 (95% CI=1.06-7.05; P=0.037) . **(Table 7)**

Table 7- Multiple logistic regressions for performance of participants in assessment of RBC morphology with associated factors

Variables	All correctly reported	Not all correctly reported	AOR (95% CI)	p-value
Experience in medical laboratory				
< 4 years	6	64		
5-8 years	14	74		
9-12 years	5	18		
>13	6	15		
Number of slides examined per day				
>10	0	1		
From 5-10	10	21	2.74(1.06-7.05)	0.037
<5	19	127		
None	2	22	1	

Therefore, No statistically significant association between performance of professionals on assessment of RBC morphology and age, educational status, experience as medical laboratory, experience in hematology laboratory, and utilization of SOP as well as job aid except number of slides examined per day was seen.

5.6.2 Multiple logistic regressions for performance of participants in assessment of WBC morphology with associated factors

For those variables which p value for Chi squire ≤ 0.2 multiple logistic regressions was calculated. No statistical significant association between performance of laboratory professionals with age, experience in medical laboratory and utilization of SOP as well as job aid. As shown in Table 8, those who were examining 5-10 slides per day were 12.34 times more likely to perform well (AOR=12.34 95% CI, 4.11-37.01; P=0.001) compared to who did not perform per day. professionals who had 4-6 years' experience in hematology laboratory were 0.06 times less likely to perform well compared with professionals who had greater than ten years' experience in hematology laboratory (AOR=0.06, 95% CI, 0.005- 0.743, P=0.028). **(Table 8)**

Table 8-Multiple logistic regressions for performance of participants in assessment of WBC morphology with associated factors

Variables	All correctly reported	Not all correctly reported	AOR(95% CI)	p-value
Age				
22-26	8	43		
27-31	13	78		
32-36	5	37		
>37	6	12		
Experience in medical laboratory				
< 4 years	14	56		
5-8 years	7	81		
9-12 years	3	20		
>13	8	13		
Experience in hematology laboratory				
<3 years	15	58		
4-6 years	1	50	0.06(0.005- 0.743)	0.028
7-9 years	5	37		
>10 years	11	25	1	
Number of slides examined per day				
>10	0	1		
From 5-10	17	14	12.34(4.11- 37.01)	P=0.001
<5	14	132		
None	1	23	1	
Utilization of sop and job aid				
Yes	30	138		
No	2	32		

Over all statistical significant association was observed on performance of professionals on assessment of WBC morphology with number of slides examined per day and experience in hematology laboratory. Conversely, No statistical significant association between performance of professionals on assessment of WBC morphology and age, educational status, experience as medical laboratory and utilization of SOP as well as job aid.

5.6.3 Multiple logistic regressions for performance of participants in platelets estimation and associated factors

For those variables which p value for Chi squire ≤ 0.2 multiple logistic regressions was computed. There was no statistical significant association between performance of laboratory professionals in platelet estimation with educational status, experience in medical laboratory. Whereas those professionals who had 4-6 years' experience in hematology laboratory were 0.027 less likely to perform well compared with professionals who had greater than ten years' experience in hematology laboratory (AOR=0.027, 95% CI, 0.002- 0.329, P=0.005). **(Table 9)**

Table 9-Multiple logistic regressions for performance of participants in platelets estimation and associated factors

Variable	All three slides correctly estimated	Not all slides correctly estimated	AOR (95% CI)	P value
Educational status				
Diploma	0	31		
BSC(generic)	11	94		
BSC(post basic)	10	48		
Masters	2	6		
Experience in medical laboratory				

< 4 years	6	64		
5-8 years	8	80		
9-12 years	4	19		
>13	5	16		
Experience in hematology laboratory				
<3 years	4	69		
4-6 years	0	51	0.027(0.002-0.329)	0.005
7-9 years	9	33		
>10 years	10	26	1	

5.7 Inter-rater agreement between two reference readers in assessment of RBC, WBC morphology and platelet estimation.

Inter-rater agreement between two reference readers (two pathologist) in assessment of RBC morphology, WBC morphology and platelet estimation for each set of abnormal and normal morphology was in strong agreement with kappa= 1. **(Table 10)**

Table 10- Inter-rater agreement between two reference readers in assessment of RBC, WBC morphology and platelets estimation

RR2 (RBC,WBC and platelets)	RR1 (RBC,WBC and platelets)				
	Abnormal	**Normal**	**Total**	**Agreement**	**Kappa**
Abnormal	2424	0	2424	100%	1
Normal	0	606	606		
Total	2424	606	3030		

NB; RR=reference reader

5.8 Inter-rater agreements between participants reading and references reading in RBC, WBC morphology and platelets estimation

The overall agreements between references reading and participants reading in assessment of RBC morphology and WBC morphology was 55% with kappa=0.12 and 52% with Kappa value of 0.02 respectively. Kappa value of 0.12 and 0.02 showed slight agreement between them. Kappa value of less zero was found between participants reading and references reading in platelet estimation which indicated, less than chance based on the cut off proposed by Landis and Koch as standards for strength of agreement of Kappa coefficient. (**Table 11**)

Table 11-Inter-rater agreements between participants reading and references reading in RBC, WBC morphology and platelets estimation

Cell types	Participants reading	RR				
		Abnormal	**Normal**	**Total**	**Agreement**	**Kappa**
RBC	**Abnormal**	205	76	281	55%	0.12
	Normal	199	126	325		
	Total	404	202	606		
	Participants reading	**RR**				
		Abnormal	**Normal**	**Total**	**Agreement**	**Kappa**
WBC	**Abnormal**	835	89	924	52%	0.02
	Normal	781	113	894		
	Total	1616	202	1818		
	Participants reading	**RR**				
		Abnormal	**Normal**	**Total**	**Agreement**	**Kappa**
Platelets	**Abnormal**	204	157	361	41%	-0.26
	Normal	200	45	245		
	Total	404	202	606		

***NB**: RBC morphology panel slides include; Slide 6 (anisocytosis), Slide 6 (pokilocytosis) and Slide 10 (normocytic normochromic).*

WBC panel slides include; slide1 (hyper-segmented neutrophils), slide2 (blast cells), slide 3(lymphocytosis), slide5 (promylocyte), slide7 (blast cells), slide 8(leukocytosis), slide 8(blast cells), slide 9(leukopenia) and slide10 (mature WBC with normal count).

Platelets estimation slides include; Slide1 (thrombocytosis), Slide 9 (thrombocytopenia) and Slide 10(normal platelet count)

5.9 Inter-rater agreement between participants reading and malaria microscopist reading on malaria detection from RBC inclusion (malaria) slide

Agreement between malaria microscopist reading and participants reading was 35% with kappa=0 showed, slight agreement based on kappa index interpretation**. (Table 12)**

Table 12-Inter-rater agreement between participants reading and malaria microscopist reading on malaria detection from RBC inclusion (malaria) slide

Participants reading	**RR**				
	Positive	**Negative**	**Total**	**Agreement**	**Kappa**
Positive	70	0	70	35%	0
Negative	132	0	132		
Total	202	0	202		

6. Discussion

6.1 performances of laboratory professionals on MAPBCM

Evaluating blood cells morphology by microscope is standard laboratory method to diagnose different forms of anemia, leukemia and used to detect quantitative and qualitative abnormalities of WBC,RBC and platelets, it can be ordered by physicians or laboratory professionals himself/herself to verify results generated by automated hematology analyzers as quality control tool but interpretation of results need professional experts.

This study is the first of its kind in assessing performance of medical laboratory professionals on peripheral blood morphology microscopy in Ethiopia. In this study, among the participants, only, 31(15.3%) correctly reported all RBC morphology panel slides, 32 (15.8%) correctly reported all major WBC morphology panel slides and 23(11.4%) estimated platelet correctly from all three platelets estimation panel slides.

On RBC morphology slides 134 (66.3%) of participants were recognized anisocytosis and 71(35.1%) reported pokilocytosis other than tear drop cells correctly. Likewise, 31(15.3%) of participants could recognized tear drop cells. Though similar studies to compare our finding are hardly available, the most probable explanation could be that attention is being given more to automated hematology with continuous refreshment trainings as a result of the donor supported HIV antiretroviral therapy program. The fact that none of the participants of this study had in service training on peripheral blood morphology evaluation, to fill their gap of pre-service trainings, supports our speculation. Studies have indicated the more peripheral smear review professionals undertake the more they are able to identify abnormalities correctly (24).

Our finding on WBC morphology assessment indicated, only 81 (40.1%) of participants were familiar with hyper segmented neutrophil. Myeloblast (myeloid precursors) was failed to recognized by 156(77.2%) and 136(67.3%) of participants from AML and CML cases respectively. About 91(45%) and 74(37%) of participants was reported myeloblast of case of AML and CML as other blast cells respectively. This finding of high failure was incomparable with similar study conducted in hospitals of New Orleans city in the state of Louisiana where 5% of participants failed to recognize myeloid precursors (27). This could possibly be due to quality

of practical pre-service trainings with more exposure to well characterized abnormal slides in their well-developed health facilities, frequency of peripheral smear evaluations in their health facilities, and magnitude of leukemia in their setting which expose professionals to frequent slide reviews.

In the current study 179(88.6%) of participants were under-looked lymphoblast which was very high compared to study conducted in New Orleans city hospitals in the state of Louisiana , 23% of participants failed to recognize lymphoblast. Moreover, 89(44.1%) failed to report normal WBC slides from healthy person as normal in this study; this is also too high compared to the previous study of 3% failure (27).

Participant readings on RBC inclusion (malaria) were also observed in this study. Of the participants, 70(34.7%) were able to detect malaria, 58(28.7%) were able to report including of species as *Plasmodium falciparum* correctly. Agreement between malaria microscopist reading with participants reading in detection of malaria was very low 35% (kappa=0) compared to findings of Alemu M *et al* from Tigray Region with malaria panels of six slides of 79% (kappa=0.62)(28). The finding of this study also incomparable with other study conducted in Ethiopia, among professionals of defense health facilities in Addis Ababa and its surrounding Areas, agreement between reference readers and participants in detection of malaria parasite was 71.4% with kappa = 0.4 from six stained malaria panels (29). A better agreement 88%with Kappa=0.76 for malaria detection and kappa=0.63 for species level identification was reported by Ayalew F *et al* which studied medical laboratory professional working in eleven health facilities in Hawassa city, Southern Ethiopia (38). The observed low agreement may be associated with lack of consciousness to detect malaria from blood morphology request. The other studies are specifically meant for performance evaluation of malaria microscopy and professionals are expected to detect presence or malaria and absence as well as species identification (28, 29, 38). Lack of skill as well as experience on malaria microscopy may also be the possible reason for low agreement between malaria microscopist and participants

2.2 Factors associated with the performance of professionals on MAPBCM.

Overall agreement between reference readers and participants on assessment of peripheral blood cells was 0.12, 0.02% and -0.26 for RBC, WBC and platelets, respectively. This is defined as '*slight*' for both RBC and WBC since both are between 0.00-0.20. Kappa value for platelets were less than zero (-0.26) which means '*poor*' based on the Kappa index of Landis and Koch shows less than even chance (36, 37). Since there was no one who had training on MAPBCM, association between training status of participants and performance of professionals on MAPBCM were not computed even though, over all low agreement may indicate lack of training and potential association between them.

Statistically significant association was noted between experience of professionals in hematology laboratory with performance of professionals in assessment of WBC morphology (p=0.028) and platelet estimation (p=0.005). Similarly Statistical significant association was observed between number of slides examined per day with performance of professionals on assessment of WBC morphology (p=0.001), and RBC morphology (0.037), professionals who were examine more morphology slides per day in routine work had good performance compared to less. This finding clearly indicates with more exposure on identification of abnormal slides, medical laboratory professionals also perform well and hence underscoring for the need for continuous practical exposure. This finding was comparable to study conducted by Brereton M. participants who regularly delivered morphological report, identified more relevant morphologic feature and had overall rate of better diagnose (24).

In the current study educational back ground had no association with performance of laboratory professionals on assessment of cell morphology. This is different from study conducted in Walden University where significant discrepancy in the levels of accuracy between clinical laboratory scientists and clinical laboratory technicians were found out (30). This suggests the need for improving our medical laboratory science curricula at all levels to focus on practical aspects.

8. Strength and Limitation of the study

8.1 Strength of the study

This study is the first of its kind in the country and helps as a baseline information to the various stakeholders (including health facilities, Ministry of health (MOH), higher education institutions teaching medical laboratory professionals) to take appropriate action

8.2 Limitations of the study

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 MAPBCM since all participants may be well aware that they were examining competency assessment slides. Moreover it was difficult to know professionals performance on smearing and staining of blood smear for cell morphology assessment.

The second limitation of this study was lack of adequate literature to make appropriate comparison of the finding.

9. Conclusion

Few participants were correctly reported all RBC morphology, WBC morphology and platelets estimation panel slides. Experience of professionals in hematology laboratory had statistical significant association with performance of professionals in assessment of WBC morphology and platelet estimation. Number of slides examined per day had statistical significant association with performance of laboratory professionals on assessment of RBC and WBC morphology. No statistical significant association was seen between performance of professionals on assessment of RBC morphology and age, educational status, experience as medical laboratory professional, experience in hematology laboratory and utilization of SOP as well as job aid. Performance of professionals on platelet estimation and WBC morphology assessment had no statistical significant association with age, educational status, experience as medical laboratory and utilization of SOP as well as job aid. The overall low agreement between references reading and participants reading was found for RBC morphology, WBC morphology, platelet estimation and malaria detection, respectively.

10. Recommendation

- ✓ The lower agreement of the professionals with expert in MAPBCM should be addressed through training by hospital administrators, quality officers, national and regional laboratories.
- ✓ Continuous assessment and supervision have to be done by hospital administrators, quality officers, national and regional laboratories in order to check the performance of laboratory professionals working on this area.
- ✓ Giving frequent training to improve skills in identification and recognition of cell morphology.
- ✓ Since no statistically significant association was noted between performance of professionals and education level. However, performance had an association with experience in hematology laboratory hence revisiting the medical laboratory science curricula focusing more on the practical aspect is recommended

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Annex 1: Wright’s staining principle

Wright's stain is a combination of methylene blue, cationic or basic dye and eosin, an anionic or acidic dye. A cationic or basic dye (methylene blue or its oxidation products such as azure B), binds to anionic sites and gives a blue color to nucleic acids, proteins of the cell nuclei, cytoplasm of the immature cells, granules of basophils and weakly to granules of neutrophils. An anionic or acidic dye such as eosin Y or eosin B on the other hand binds to cationic sites on proteins and gives an orange-red color to hemoglobin molecules and eosinophil granules (35).

Annex 2: Sampling frame of study participants from each participant hospitals

No	Name of hospitals	Number of laboratory professionals	Workstation assigning	Number of laboratory professionals in peripheral blood morphology workstation	Proportion	sample size
1	TASH	60	Permanent bases	10	-	9
2	St. Paul hospital millennium medical collage	48	Permanent bases	6	-	3
3	St. peters specialized hospital	41	Rotation Bases	41	0.16	28
4	Amanuel mental specialized Hospital	39	Rotation bases	39	0.15	27
5	All African leprosy ,tuberculosis rehabilitation training center	25	Permanent Bases	7	-	7

6	Federal armed force Hospital	35	permanent bases	6		6
7	Federal prison Hospital	8	Rotation bases	8	0.03	6
8	Yekatit 12 Memorial Hospital	37	Rotation bases	37	0.15	26
9	Ras Dest Damtaw memorial Hospital	34	Rotation bases	34	0.13	23
10	Zewditu Memorial Hospital	34	Rotation bases	34	0.13	23
11	Dagmawi Miniliik Memorial Hospital	22	Rotation bases	22	0.09	16
12	Gandi Memorial hospital	16	Rotation bases	16	0.06	11
13	Tirunesh Beijing general hospital	23	Rotation bases	23	0.1	17
Total	13	422		283	1	202

Annex 3: Information sheet

1. Background to the study

Examination of properly prepared and stained slide by skilled microscopist has been set as gold standard for identification of peripheral blood morphology. 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 peripheral blood morphology led to the inappropriate administration of drugs. No result is better than poor result.

2. Purpose of the Research Project

Evaluating the performance of laboratory Professionals on manual assessment of peripheral blood cells morphology and associated factors helps to identify any gaps which leads to miss diagnosis and also helps to know how to improve the performance of the laboratory professionals.

3. Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Then you will be provided with panel of ten slides for peripheral blood morphology examination. You will review them and report on the report format; you will be given 5 minutes per slide. In addition, there will be a self-administered structured questionnaire including information on the socio-demographic characteristics, educational background, work experience, average number of slides examined per day, training (in service) and availability and usage of SOP, your skill of identifying and reporting abnormal peripheral blood morphology, which will take about 5 minutes.

4. Potential risks and Discomforts

There is no risk related to participating in the study.

5. Confidentiality

The information collected will not have any specific information including name that might break your anonymity. You are also not forced to tell anything you don't want to answer regarding yourself. The information we will collect will be kept in a locked file cabinet, or be protected by a password.

6. Potential benefits to subjects and/or to the society

You will not receive any payment for participating in this study as compensation. However, your participation will help to identify gaps for improving peripheral blood morphology analysis. As medical laboratory professional, your involvement for this quality improvement effort will give you professional satisfaction as the evidence based interventions will in turn help you to improve the hematology laboratory service. Hence, you are indirectly benefiting patients you are serving, the clinicians managing the patients and the society at large in this respect.

7. Participation and Withdrawal from the Study

You have the right not to participate in the study or can with draw from the study any time.

8. Contact information

If you have any questions, you may consult the PI or supervisors by the following addresses:

Yeshi Yimer (PI)

Department of Medical Laboratory Sciences, College of Health Science

Addis Ababa University

Addis Ababa, Ethiopia.

Email: yeshiyimer19@gmail.com

Tel: +251947754031

Dr Aster Tsegaye 0911 696085

Fatuma Hassen 0911418062

Department of Medical Laboratory Science Research and Ethics Committee 011 2755170

Annex 4. Consent form

I have been informed that the objective of the study is to evaluate the performance of laboratory Professionals on manual assessment of peripheral blood cells morphology and associated factors which will help to identify any gaps which leads to misdiagnosis and also helps to develop job aids and SOPs to improve the performance of the laboratory professionals.

I understood that the study is useful without any health risks to me and agreed voluntarily to participate in the study.

Signature of participant: _____Date _____

Annex 5- consent form for blood donors

Purpose

My name is Yeshi Yimer and I want to develop a collection of blood morphology slides for the purpose of evaluating the performance of laboratory professionals in manual assessment of blood cells morphology .So I request to collect a blood samples to make large number of identical blood smears.

Procedures

I will have a 3 ml.–5 ml. sample extracted for this study. The blood will be assessed by microscopy to check the abnormalities of cells and at the same time check with blood cell analyzer. If it is comparable and found as what I want, it will be placed on slides and used for evaluating the performance of laboratory professionals working in thirteen government hospitals of Addis Ababa.

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: Yeshi Yimer (PI) Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University

Addis Ababa, Ethiopia.

Email: yeshiyimer19@gmail.com

Tel: +251947754031

Annex 6: Questionnaire

Health Facility Name: _____

Participants Code Number:_____

Code	Question	Response Category	Remark
101	Age in Years		
102	Sex	1.male 2.female	
103	Year of Service as medical laboratory professional	------ years	
104	What did you graduate with?	1.Diploma 2.BSc (generic) 3. BSc (Post Basic) 5. Masters	
105	How long has it been since you Started working on manual assessment of peripheral blood cells morphology?	_____ years	
106	How many peripheral blood morphology slides do you Examine daily?	1. Less than 5 2. From 5-10 3. More than 10 4. None	
107	Have you ever had an in-service	1. Yes 2. No	

	Training on manual assessment of peripheral blood cells?		
108	If yes, how many times?	----------	
109	Do you use color atlas, job aid and SOP during manual assessment of peripheral blood cells morphology?	1.Yes 2.No	
110	How frequent does your laboratory receive peripheral blood morphology request?	---------per day ----------per month	
111	Do you perform peripheral blood smear review for flagged automated hematology results?	1. Always 2. Sometimes 3. Never	
112	What will you do if you find a cell that you are unable to identify on a peripheral blood smear?		

Annex 7: Observational checklist

Health Facility Name: _____

Date of observation:_____

1. Availability of Job aid 1. Yes 2. No

2. Availability of SOP 1. Yes 2. No

3. Any peripheral blood morphology report by the laboratory 1. Yes 2. No

4. Check if lab personnel are working on rotation basis or permanently assigned (Permanent in hematology Lab 1. Yes 2. No); if rotation, check when was the last time the lab personnel working in Hematology lab and for how long

5. Check records for microscope maintenance 1.yes 2.No

6. Availability of sufficient reagents 1.yes 2.No

Annex -8 log sheets

Result reporting form for laboratory professionals

Name of health facility_____

Code Lab technicians/technologist _____

Slide ID	**Result**			
	RBC(size, shape, Hgb content ,arrangement, inclusion bodies)	✓ **WBC Estimate** ✓ **Diff. count by considering presence of immature cells including stage of maturation**	✓ **Platelet Estimate**	**Diagnose(if possible)**
001				
002				
003				
004				
005				
006				

007				
008				
009				
010				

Annex 9: Checklist to check microscope quality through quality control slide of mature cells

Name of hospital	**Component to be checked**		**Color**	**Yes/no**
	Nucleus of cells		Purple-violate	
	Cytoplasm	Erythrocyte	Dark pink	
		Lymphocyte	Blue	
		Monocyte	Gray-blue	
		Neutrophile	Pink/orange	
		Basophile	Blue	
	Granule	Neutrophile	Purple	
		Basophile	Purple black	
		Eosinophile	Red-orange	
		Platelet	Purple	

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Yeshi Yimer Abebe (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Fatuma Hassen (MPH, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor:

Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia