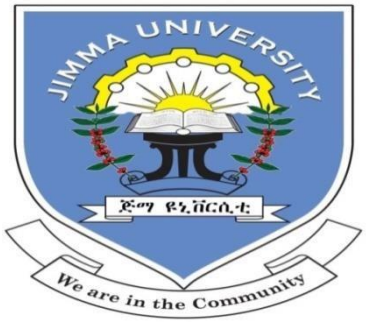
CORRELATION OF MANUAL PERIPHERAL BLOOD SMEAR EXAMINATION WITH RBC INDICES AND HISTOGRAMS OBTAINED FROM BECKMAN COULTER UNICEL DXH-800 AUTOMATED HEMATOLOGY ANALYZER IN ANEMIC PATIENTS AT JIMMA MEDICAL CENTER, SOUTHWEST ETHIOPIA



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JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCE

SCHOOL OF MEDICAL LABORATORY SCIENCE

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ABSTRACT

Background: *The manual peripheral blood smear examination is a comprehensive examination of the blood film to detect clinically significant abnormalities in leukocyte, erythrocyte, and platelet morphology. It is a time-consuming technique, but it is a more cost-effective and sensitive technique than an automated hematological analyzer. On the other hand, the automated hematology analyzer is faster, more objective, and reduces labor cost but cannot reveal the variety of abnormal cells. This study aims to find out the correlation of peripheral blood smear examination with RBC indices and histograms obtained from an automated hematological analyzer in diagnosis and morphological typing of anemia.*

Objective*: To compare the finding of the manual peripheral blood smear examination with red cell indices and histograms obtained from the Beckman coulter UniCel DxH-800 automated hematological analyzer in anemic patients.*

Method*: A comparative cross-sectional study was conducted by using a convenient sampling technique. A total of 250 blood samples were analyzed from July 25- Oct 25, 2022 at Jimma medical center. About 3 ml of blood samples were collected for the analysis into an EDTA anticoagulated tube. Kappa statistics were used to measure the agreement between the two methods. The final results were presented by tables and figures.*

Result: *The manual peripheral blood smear examination revealed that the predominant morphological typing of anemia was microcytic hypochromic anemia (45.2%), while normocytic normochromic anemia (48.0%) was the most common anemia based on red cell indices and histogram patterns obtained from the automated hematological analyzer. The sensitivity and specificity of the red cell indices and histograms were 85.8% and 94.1% for microcytic hypochromic anemia, 91.6% and 84.6% for normocytic normochromic anemia and 91.7% and 98.7% for macrocytic normochromic anemia respectively.*

Conclusion and recommendation: *There was a statistically significant difference (p=0.000) between the manual peripheral blood smear examination and the automated hematological analyzer. A manual peripheral blood smear examination should be used in addition to the automated hematological analyzer for a better diagnosis and management of anemia.*

Keywords*: Histograms, Peripheral blood smear, Red cell indices*

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Abbreviation and Acronym

CBC Complete blood count

CLSI Clinical and Laboratory Standard Institute

EDTA Ethylene diamine tetra-acetic acid

EPO Erythropoietin

GC Gregorian calendar

HCT Hematocrit

HGB Hemoglobin

JMC Jimma Medical Center

MCH Mean corpuscular hemoglobin

MCHC Mean corpuscular hemoglobin concentration

MCV Mean cell volume

NPV Negative predictive value

PBS Peripheral blood smear

PCV Packed cell volume

PLT Platelet

PPV Positive predictive value

RBCs Red blood cells

RDW Red cell distribution width

SOP Standard operating procedure

SPSS Statistical package for social science

WBCs White blood cells

WHO World health organization

1. INTRODUCTION

1.1. Background

Anemia is a reduction in the total number of red blood cells or hemoglobin in the blood, resulting in a condition in which the red blood cells do not transport enough oxygen to the body's tissues (1,2). It affects more than 2 billion individuals worldwide, accounting for 30 percent of the world population, and preschool children and pregnant women were mostly affected globally (3).

The investigation of anemia can be performed by either a manual procedure, such as manual hemoglobin, manual hematocrit, and manual peripheral blood smear examination or an automated hematological analyzer, and the microscopic examination of a peripheral blood smear provides the clinician with a lot of information (4). Morphologically, anemia can be classified as microcytic, normocytic, or macrocytic based on red blood cell (RBC) size (5).

The peripheral blood smear examination is a comprehensive examination of the blood film to detect clinically significant abnormalities in leukocyte, erythrocyte, and platelet morphology and it is an appropriate diagnostic test in a patient with a suspected primary hematologic disorder or with unexplained leukocytosis, leukopenia, anemia, polycythemia, thrombocytopenia, or thrombocytosis (6).

A peripheral blood smear examination can reveal an abnormal red cell shape, which can be used to diagnose several types of anemia; the red cell morphology evaluation should involve an inspection for deviations in size, shape, distribution, hemoglobin content, and the appearance of inclusions (7). Furthermore, a peripheral blood smear examination can confirm "flagged" results from an automated hematology analyzer (6).

The automated hematological analyzer is faster, more objective, and free of the subjective errors that occur with the manual peripheral blood smear examination (8). As a result, the automated hematology analyzer has improved accuracy, can analyze a higher number of samples with more speed and precision, and reduces the need for human intervention in test entry, sampling, sample dilution, and analysis (9).

The Beckman Coulter UniCel DxH-800 automated hematology analyzer is a scalable, fully automated hematology analyzer system capable of analyzing up to 100 samples per hour and providing a complete blood count (CBC), 5-part white blood cell (WBC) differential, nucleated

RBC count, and reticulocyte count from 165 μL of sample (10). It works based on the impedance principle, accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture; as each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture and this causes a measurable electronic pulse in which the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume (10).

Coulter instruments typically have two measurement channels in which RBC, WBC, and hemoglobin are considered to be measured directly and the RBC mean cell volume (MCV) is the average volume of the RBCs taken from the volume distribution data and the hematocrit (HCT), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) are calculated from measured and derived values (11). The results of automated hematology analyzers are shown as a histogram and figures (12).

Red blood cell indices are useful parameters when investigating anemia and they provide a general idea of the clinical picture, predict the red blood cell appearance, and aid in the classification of anemia and calculated using the red blood cell count, hematocrit, and hemoglobin values generated by automated hematology analyzers, or directly measured in the case of MCV, depending on the model of instrument being used (13).

The histogram is a graph that shows the frequency of cells in relation to their size; it is an important component of the automated hematology analyzer, and when combined with red blood cell indices, hematocrit, and red blood cell distribution width, it can help in the diagnosis and management of anemia (7,14). Red cell distribution width (RDW) is the coefficient of variation, or standard deviation, of the MCV and is used to predict the degree of red blood cell size variation, known as anisocytosis and an increase in the RDW indicates the presence of anisocytosis on the peripheral blood smear (13).

The automated hematology analyzer reduces the labor cost but creates decision making problem to finalize the diagnosis and cannot reveal the variety of abnormal cells (15,16). On the other hand, peripheral blood smear examination is time-consuming and prone to inter-observer

variation but it is a more cost-effective and sensitive technique than an automated hematological analyzer (5).

In most automated laboratories, peripheral blood smear examinations are limited to cases where the instrument "flags" the potential presence of abnormal cells or where findings may interfere with analysis and in cases when there is clinical suspicion of leukemia, an examination of the peripheral blood smear is required to make the presumptive diagnosis (17).

Peripheral blood smear review is a useful and economical diagnostic tool that can be used in the investigation of hematological disorders, and it provides rapid, reliable access to information about a variety of hematologic disorders; in some cases, review of peripheral smears along with clinical data may be sufficient to establish a diagnosis (18).

Peripheral blood smear examination, in addition to CBC performed by automated hematology analyzers, can produce a more accurate result by identifying different types of anemia, and smear examination also aids in the cross-checking of RBC indices and histograms obtained from an automated hematology analyzers (19).

1.2. Statement of the problem

In modern hospital and clinical settings, the automated hematology analyzer with complete blood count results has become the cornerstone of modern laboratories and has replaced the manual methods for common hematological parameters (20). In most institutions, the practice of reviewing all automated hematology analyzer results by preparing a stained blood film for microscopic examination is no longer practiced (15).

Reducing the number of manual scans of peripheral blood smears for the result of an automated hematological analyzer increases the release of less accurate results, which has a detrimental impact on the diagnosis and treatment of anemia (5). The manual peripheral blood smear examination aids in the diagnosis of anemia, and the advantage of a manual scan is that it can discover clinically significant morphological abnormalities and inclusions (pencil cells, sickle cells, teardrop cells, schistocytes, basophilic stippling, Pappenheimer bodies, Heinz Bodies, Howell-Jolly bodies, and so on) that the automated analyzer cannot detect (5).

The global prevalence of abnormal blood cell morphology is increasing due to a variety of factors. A study conducted in Miami, USA, found that 96% of people with sickle cell trait had abnormal RBCs (21). In Germany, 84%, 98%, and 98% of COVID-19 patients have RBC, WBC, and platelet morphological abnormalities, respectively (22). These findings suggested that the examination of peripheral blood smears is an important part of laboratory procedure in the inspection of abnormal red cell morphology (23).

Automated hematology analyzers are the most important instruments in today‘s clinical laboratory, able to perform thousands of CBCs per day in a completely automated manner. However, in some cases, it requires operator intervention or a confirmatory test, such as a peripheral blood smear review and a manual differential cell count, and these additional steps impact laboratory turnaround time, efficiency, and labor costs, which have a negative impact on patient management (10).

The inability of automated hematology analyzers to reliably distinguish cells from other particles or cell fragments of the same volume, e.g., fragmented RBCs may be counted as platelets, resulting in an incorrectly increased platelet count and decreased WBC and RBC counts, and larger platelet clumps may be counted as WBCs, resulting in a falsely decreased platelet count and potentially an increase in WBC count, resulting in an inaccurate diagnosis and impact the diagnosis and clinical management of various hematological disorders (11).

The PBS examination and an automated hematology analyzer have shown some discordance in the investigation of anemia. The PBS examination revealed additional information in 11.4% and 13.9% of cases in India and Israel, respectively (24,25). Furthermore, in Malawi, 69.5% of PBS examinations provided additional information to CBC, indicating an automation error (26) . As a result, the PBS examination provides the most information at the lowest cost and can be used to support healthcare systems in both developing and developed countries (27).

Neglecting PBS morphological examination has a negative impact on the management of hematological disorders. According to Beckman et al. 2020, potentially added clinical value would have been missed in 23% of cases if the PBS examination was not performed (29). According to a study in India, ignoring PBS examination has resulted in misclassification of

anemia and a misclassified anemia due to ignoring the PBS examination implies the risk of prolonging avertable patient suffering (24).

The PBS examination is important in addressing health issues, particularly in the investigation of hematological disorders and its role in providing both supportive and unique information to the complete blood cell count rendered PBS examination irreplaceable by modern automated hematology analyzers (27).

Despite its numerous benefits, the PBS examination service has a low utilization rate. Manual PBS examination was found to be 16.2% in the United States and 22.4% in Thailand (28,29). PBS examination was performed on only 39.2% of eligible specimens in Malawi public hospital (26). Furthermore, the developing country has limited access to automated hematology analyzer, making it difficult to deal with hematological problems in the area and in Africa, diseases control is impeded by inadequate laboratory services (30).

In a study conducted in Ethiopia, the rate of peripheral blood smear examination was very low among general hospitals and district hospitals, which was 14.9% and 6.5%, respectively and patient outcomes were delayed due to a lack of strengthened PBS examination services (27). Hence, this alarms us to assess the correlation of the PBS examination with the automated hematology analyzers in the study area, which lacks specific data despite the heavy burden of anemia in the area.

Generally, in most institutions, there is no practice of reviewing the results of an automated hematology analyzer with a manual peripheral blood smear examination, which results in the release of less accurate and reliable results that have an impact on the diagnosis of various hematological disorders. Method comparison can play an important role in improving the quality of laboratory services and guiding the right clinical decisions. So this study aims to find out the correlation of the manual peripheral blood smear examination with RBC indices and histograms obtained from the Beckman Coulter UniCel DxH-800 automated hematological analyzer in anemic patients.

1.3. Significance of the study

The morphological typing of anemia by incorporating peripheral blood smear examination with RBC indices and histograms obtained from an automated hematological analyzer is very useful in the diagnosis and treatment of anemia.

The findings of this study will help clinicians, to request and use a method that provides them with a lot of information and makes their diagnosis easier. This study's findings will also benefit laboratory professionals by reducing ambiguity, interpersonal variation, and decision-making challenges that arise during the diagnosis and morphological typing of anemia. The finding will also help the study participants and the community, by providing a method that produces accurate and correct results during the diagnosis of anemia so that they can get better treatment. It will also allow policymakers to implement a more accurate and precise method for the diagnosis and morphological typing of anemia. It will also serve as baseline data for future studies.

2. LITERATURE REVIEW

Although automated hematology analyzers are now employed in almost all large diagnostic and clinical settings, peripheral smear examination has always been the foundation of diagnostic hematology. Despite the sophistication of modern instruments, microscopic examination is required for initial calibration as well as for the presumed diagnosis of anemia, leukemia, and other associated disorders (15).

A cross-sectional study conducted in Banglore, India, from August 2014 to July 2015 by Sandhya V. *et al,* to determine the correlation of peripheral blood smear with red cell indices and histograms showed a statistically significant result ($p<0.0001$) between the two methods and there was a moderate (51.8%) agreement between the two methods. In addition, microcytic hypochromic anemia was the predominant anemia found, with the prevalence of 46%, followed by normocytic normochromic anemia, which was seen in 21.8% of cases by peripheral smear examination (31).

Another cross-sectional study conducted in Maharashtra, India, in 2019 by Bhatt N *et al* , to determine the correlation of peripheral blood smear with red cell indices and histograms in the diagnosis of anemia yielded statistically significant results with a P-value of <0.001. Furthermore, microcytic hypochromic anemia was shown to be the most frequent anemia, with a prevalence of 64.77%, followed by normocytic normochromic anemia, which was seen in 19.31% (32).

Another similar cross-sectional study conducted in Maharashtra, India, in 2018 by Jain A *et al*, to compare the peripheral blood smear with an automated hematology analyzer in anemic patients, microcytic hypochromic anemia was the predominant anemia 40% of cases followed by normocytic normochromic anemia in 20% of cases, and 10% of cases showed macrocytic anemia by the manual PBS examination. There were 44.4% microcytic hypochromic anemia, 18.8% normocytic normochromic anemia, and 16% macrocytic normochromic anemia by the automated hematological analyzer (33).

In a cross-sectional study conducted in Morang, Nepal, from January to June 2019 by Singh M *et al,* to compare peripheral blood smear findings with an automated hematology analyzer generated red cell parameters; the most common anemia seen on smear examination was

microcytic hypochromic anemia with 60% of cases followed by dimorphic anemia in 20.86% of cases. The sensitivity of MCV, MCHC, and MCH was 78%, 14%, and 80% respectively in the detection of microcytic hypochromic anemia. The sensitivity of MCV and MCH was found to be 100 % in the detection of macrocytic anemia. The sensitivity of MCV, MCHC, and MCH was 78%, 100%, and 67% respectively in the detection of normocytic normochromic anemia (19).

In a cross-sectional study conducted in Gujarat, India, in 2020 by Jansari T *et al*, to compare the manual PBS examination with an automated hematology analyzer, microcytic hypochromic anemia (53.8%) was the most common type, followed by normocytic normochromic anemia (36.9%) by PBS examination. More cases (57.6%) were also microcytic hypochromic type, followed by normocytic normochromic (30.7%) by an automated hematology analyzer. The sensitivity and specificity of red blood cell indices and histogram were 94.2% and 63.1%, 96.7%% and 49.1%, 98.2% and 90.3% for microcytic hypochromic, normocytic normochromic and macrocytic anemia respectively (34).

In a comparative cross-sectional study done in Pakistan, in 2013 by Farah E *et al*, to compare automated hematology analyzer with peripheral blood smear, the study showed statistically significant differences (p-value < 0.001) for an automated hematology analyzer and peripheral blood smear. From the total of screened individuals, the concordance between the automated hematology analyzer and visual examination of peripheral blood smear was 78% (5).

In a cross-sectional study conducted in Kerala, India, in 2019 by Venukumar M *et al*, to compare the red cell indices with the PBS examination in morphological typing of anemia, the majority of the cases were microcytic hypochromic anemia 46% followed by normocytic normochromic anemia 39% typed using peripheral smear examination. About 87.75% of cases showed concordant typing and 12.25% of cases showed non-concordant typing. The sensitivity and specificity of the automated analyzer were 97% and 91%, 96.5% and 97.5%, and 91% and 90.5% for microcytes, macrocytes and normocytes respectively (35).

In another cross-sectional study conducted in Jammu, India, from November 2017 to October 2018 by Hafiz F *et al* on the study of the morphological patterns of anemia, the majority of the patients (45%) had a mild degree of anemia with haemoglobin levels more than 9gm/dl. The

majority of the patients (40%) showed normocytic normochromic anemia followed by microcytic hypochromic anemia seen in 29% of cases by the PBS examination (4).

In another similar cross-sectional study conducted in Tamil Nadu, India, in 2018 by Samly D *et al*, on comparison of hematological parameters obtained from an automated analyzer with peripheral smear in diagnosis of anemia, 44.5% of the cases showed severe anemia followed by moderate in 42.8% and mild in 12.7% cases. The most common morphological type of anemia was normocytic normochromic anemia (57%) followed by microcytic hypochromic anemia (44%) (36).

In a cross-sectional study conducted in Uttar Pradesh, India, from June 2014 to August 2015 by Singhal S *et al*, to compare the manual PBS examination with an automated hematology analyzer, microcytic anemia (43.8%) was the commonest anemia found followed by normocytic anemia (36.5%) by the automated analyzer and similarly microcytic anemia were the commonest anemia found (49.8%) followed by normocytic anemia (36.5%) by the PBS examination. Discordance in typing of anemia between two the methods was found only in 11.4% of cases (8).

In a cross-sectional study conducted in Rajasthan, India, in 2018 by Choudhary S *et al*, to asses the sensitivity of Red cell histogram and CBC parameters against peripheral blood smear in various anemias, the predominant anemia found were microcytic hypochromic anemia about 52.67% followed by normocytic normochromic anemia 30.67% by the PBS examination. The concordant and discordant rates were 67% and 33% respectively. The sensitivity and specificity of RBC indices and histogram were 93.9% and 64.2%, 97.3% and 47.8%, and 98.7% and 93.3% for microcytic hypochromic, normocytic normochromic and macrocytic anemia respectively (37).

In a cross-sectional study conducted in Gujarat, India, from January 2018 to December 2018 by Shrivastava A *et al*, on the utility of RBC histogram in the daignosis of various anemias, the maximum numbers of cases were microcytic anemia (70%) followed by normocytic anemia (18%). The RBC histogram showed a normal curve (18%), left shift (29%), right shift (6%) Broad base (40%), short peak (2%) and bimodal (5%) (12).

In another cross-sectional study conducted in Nellore, India, in November 2016 by Rao B *et al*, on comparison of RBC histogram with manual PBS examination, the majority 63.63% cases

were microcytic hypochromic anemia followed by 19.54% normocytic normochromic anemia by the PBS examination. The histogram pattern showed a broad base in 37.72%, a left shift in 29%, a normal curve in 17.7%, a bimodal in 7.27%, a right shift in 5.45%, and a short peak in 2.7% cases (38).

In another similar cross-sectional study conducted in India, in 2018 by Garg M *et al*, to compare automated analyzer generated red blood cell parameters and histogram with peripheral smear; microcytic hypochromic anemia was the most common anemia 50.86%, followed by normocytic normochromic anemia by the PBS examination. Analysis by RBC indices showed 59.43% of microcytic hypochromic anemia, 32% of normocytic normochromic anemia, 3.43% of macrocytic anemia respectively (39).

In a cross-sectional study conducted in Islamabad, from November 2015 to April 2016 by Asghar R *et al,* on a comparison of peripheral blood smear examination with an automated hematology analyzer for diagnosing different types of anemia, the most common type of anemia diagnosed with the peripheral blood smear was microcytic hypochromic anemia 36.7% followed by normocytic normochromic anemia 13.3% while in the automated hematology analyzer microcytic hypochromic anemia 54.4% followed by normochromic normocytic anemia 11.1% (40).

In a comparative cross-sectional study done in India, from 2017 to 2019 by Ashok C *et al*, to compare the peripheral smear with RBC indices and histogram, the predominant anemia found was microcytic hypochromic anemia 62%, followed by normocytic normochromic anemia 18% by the PBS examination. Based on the red cell indices 53% of cases were found to have microcytic hypochromic anemia, 37% of cases showed normocytic normochromic anemia and 10% of cases were diagnosed as macrocytic anemia with an automated analyzer (41).

In a cross-sectional study conducted in Nigeria, in 2010 by Ike SO et al, to compare hematological parameters determined by an automated hematological analyzer and the manual counts, the manual blood smear report revealed normocytic normochromic in 50% of cases, microcytic hypochromic 16.6% of cases and 1.7% of cases showed macrocytic normochromic (42).

Generally, most of the literature shows a correlation between a peripheral blood smear and an automated hematology analyzer, but there are also some articles, that show some variation between the two methods in the diagnosis and morphological typing of anemia. So, the purpose of this study is to determine the relationship between peripheral blood smear examination and automated hematological analyzer in the diagnosis and morphological typing of anemia, and I couldn't find any articles reported on this topic in Ethiopia, so this study is useful to know and use the best method in the diagnosis and morphological typing of anemia in our country for a better diagnosis and management of anemia.

3. OBJECTIVES

3.1. General objectives

- To compare the finding of the manual peripheral blood smear examination with RBC indices and histograms obtained from the Beckman Coulter UniCel DxH-800 automated hematological analyzer in anemic patients from July 25- Oct 25, 2022 at Jimma medical center, southwest Ethiopia.

3.2. Specific objectives

- To determine the morphological typing of anemia using the findings of the peripheral blood smear examination and the Red cell indices and histograms obtained from the Beckman Coulter UniCel DxH-800 automated hematological analyzer.
- To correlate the finding of the manual peripheral blood smear examination with RBC indices and histograms obtained from the Beckman Coulter UniCel DxH-800 automated hematological analyzer.
- To determine the sensitivity, specificity, PPV and NPV of the red cell indices and histograms obtained from the Beckman Coulter UniCel DxH-800 automated hematological analyzer.

4. METHODS AND MATERIALS

4.1. Study area

The study was conducted at Jimma Medical Center (JMC) in southwest Ethiopia. It is located in Jimma town, 345 kilometers away from the capital city of Ethiopia, and the town has a highland climate with considerable rainfall, and a long rainy season. The town is located at an elevation of 1763 meters above sea level. It also has a latitude of 7°40'N and a longitude of 36°50'E. According to the CSA's 2007 census, the zone's total population is 2,486,155, with 1,250,527 males and 1,235,628 females (43).

Jimma Medical Center is one of the oldest hospitals in Ethiopia and it was established in 1937 by Italian invaders for the service of their soldiers. Currently it is one of the referral hospitals in southwest Ethiopia with 800 bed capacity. The service area includes Internal medicine, Surgery, Gynecology/Obstetrics, Pediatrics and child health, Ophthalmology and Psychiatry, Laboratory and Pharmacy. JMC laboratory has different departments and the hematology department is one of them. This department is equipped with different hematology analyzers and equipment used to perform different hematological tests.

4.2. Study design and period

A comparative cross-sectional study was conducted from July 25- Oct 25, 2022, at JMC, Jimma town.

4.3. Population

4.3.1. Source population

All anemic patients attended JMC during the study period.

4.3.2. Study population

All anemic patients requested for CBC analysis by Beckman Coulter UniCel DxH-800 automated hematology analyzer and meet the sample acceptance and rejection criteria according to standard operating procedure (SOP) at JMC during the study period.

4.4. Sample size and sampling technique

4.4.1. Sample size determination

According to the Clinical and Laboratory Standard Institute (CLSI) 2013 guideline, a minimum of 40 specimens are required for method comparison studies (44). By considering cost, time, and method used to conduct this study a total of 250 specimens were analyzed.

4.4.2. Sampling techniques

A non-probability convenient sampling technique was used.

4.5. Eligibility criteria

4.5.1. Inclusion criteria

- Blood samples of all anemic patients of both sexes and all age groups as per the WHO reference range were included in the study.

4.5.2. Exclusion criteria

- Leftover patient samples which were insufficient to carry out the study.
- Patient samples with an incomplete request form were excluded.

4.6. Variables

4.6.1. Dependent variables

- Agreement of the two methods in anemic patients

4.6.2. Independent variables

- Demographic characteristics
- PBS examination
- Red cell indices
- RBC Histograms

4.7. Data collection, processing, and laboratory analysis

4.7.1. Data collection and processing procedure

About 3 ml of whole blood specimens was collected by the phlebotomists into a tube containing EDTA anticoagulant from patients of all ages and sex groups for CBC analysis and transferred to the hematology laboratory department. The samples were analyzed by Beckman Coulter UniCel

DxH 800(Danaher Corporation, United states) automated CBC analyzer, simultaneously the blood smears were prepared and stained with the wright stain for peripheral blood smear examination under the microscope. The request forms were used to collect information about the patient's age, gender, and clinical diagnosis.

4.7.2. Laboratory analysis

Beckman Coulter UniCel DxH 800 (Danaher Corporation, United states)

CBC was performed using a Beckman coulter UniCel DxH 800 (Danaher Corporation, United states) automated hematology analyzer. After turning on the analyzer three levels of commercially prepared hematological cell controls (Normal, Low, and High) were run on the Beckman Coulter UniCel DxH 800 automated hematology analyzer. After these levels of controls passed the blood samples were analyzed according to standard operating procedure. Then the analyzer processed the sample automatically and the result was printed out for data analysis.

Peripheral blood smear examination

The blood smears were prepared from the residual well-mixed EDTA anticoagulated blood samples by placing a small drop (2 to 3 µl) of blood in the centerline of a slide about 1 cm from one end. The drop was spread out quickly along the line of contact at an angle of $30\text{-}45^0$. It was air dried for 2 minutes and fixed with methanol. The air-dried smear was then flooded with a wright stain for 1 minute and a buffer of the same quantity as the stain was gently added, and mixed by blowing on the surface and leaving for 5 minutes. The stain was washed off with tap water and air-dried. Finally, the morphological characteristics of blood cell were observed under the microscope usually 100x with oil-immersion magnification (40). Grading of morphological features will be found in **Annex IV**.

4.8. Data analysis and interpretation

The data was checked for completeness, cleaned, arranged and categorized manually and then entered into Epi data version 4.6. Then exported and analyzed using statistical package for social science (SPSS) version 25 software. Normality of the data was checked by Kolmogorov-smirnov and Shapiro-wilk tests. Descriptive statistics were used to express the demographic characteristics and other variables as needed of the study population. Kappa statistics were used to measure the agreement between the two methods. Sensitivity, Specificity, PPV and NPV were

computed. Chi-square test was used to know the association between the two methods. The p-value < 0.05 were considered statistically significant. The results were presented with tables and figures.

4.9. Data Quality Control and Assurance

Patient identification and labeling were done with care, and samples were collected and transported without delay. Clotted, mislabeled, inadequate, strongly lipemic and hemolyzed samples were rejected. Blood film for a peripheral blood smear examination was prepared with care by skilled medical laboratory technologists and the slides were reviewed by two trained laboratory technologist to ensure quality results. SOP and manufacturer instruction was followed during every step of the test performed. Supervision was made in every step of data collection. To avoid any clerical error, printout results that were generated by the analyzers were used and the result of a peripheral blood smear was recorded with extreme care. Then the results from both methods were confidentially documented, recorded, and analyzed using SPSS version 25.

4.10. Ethical consideration

The study was conducted after getting ethical clearance from the institutional review board (IRB) of the Institute of Health, Jimma University. An official support letter of request to conduct the study was written to Jimma Medical Center to obtain approval and carry out the study. The leftover specimen from the CBC analysis was used only for the intended purposes. All information collected was kept confidential.

4.11. Plan for Result Dissemination

The finalized paper of this study will be presented and submitted to Jimma University, Institute of Health, Faculty of Health Science, School of Medical Laboratory Science. A copy of this material will be given to the JMC laboratory and will be used to improve the laboratory‘s practice by developing a guideline. The finding will also be communicated to the hospital and respective stakeholders. The result will also be disseminated through publication in peer-reviewed local and international journals and through presenting it in relevant workshops, seminars, and scientific conferences.

4.12. Operational Definitions

Anemia- a medical condition characterized by low number of red blood cells or quantity of hemoglobin as per WHO reference range for age and sex **(ANNEX I).**

Mild anemia – hemoglobin value between 10gm/dl and 11gm/dl

Moderate anemia – hemoglobin value between 7gm/dl and 9.9gm/dl

Severe anemia – hemoglobin value less than 7gm/dl

Microcytic hypochromic- small red blood cell size less than the nucleus of small lymphocytes with decreased hemoglobin content and large central pallor area in wright-stained peripheral blood smear and Low MCV (<80fl), Low MCH (<27fl) from the result of an automated hematology analyzer.

Normocytic normochromic- Normal red blood cell size equal with the nucleus of small lymphocytes with normal red blood cell color and hemoglobin content in wright-stained peripheral blood smear and have a normal MCV (80-100fl) and MCH (27-32fl) from the result of an automated hematology analyzer.

Macrocytic normochromic- large red blood cell size greater than the nucleus of small lymphocytes with normal red cell color and hemoglobin content in wright-stained peripheral blood smear and High MCV (>100fl) and MCH (>32fl) from the result of an automated hematology analyzer.

Dimorphic - presence of two population of red cell (microcytic and normocytic or normocytic and macrocytic) in wright-stained peripheral blood smear.

5. RESULT

5.1. Demographic characteristics

A total of 250 anemic patient samples were included in the study. Out of 250 blood samples, 104 (41.6%) males and 146 (58.4%) females samples were analyzed. The majority of the participant age was between 15-29 years, which accounts for about 93 (37.2%), followed by <14 years, which accounts for about 79 (31.6%) **(Table 1).**

Table 1: Demographic characteristics of the study population at Jimma medical center, southwest Ethiopia, October 2022

Variables		**Frequency**	**Percent (%)**
Sex	Male	104	41.6 %
	Female	146	58.4 %
	Total	250	100 %
Age	<14	79	31.6 %
	15-29	93	37.2 %
	30-44	51	20.4 %
	45-59	12	4.8 %
	>60	15	6.0 %
	Total	250	100 %

5.2. Severity of anemia based on hemoglobin level

Based on the hemoglobin level obtained from the automated hematological analyzer, out of the 250 anemic blood samples analyzed, 111 (44.4%) were severe anemia, followed by 92 (36.8%) moderate anemia **(Table 2).**

Table 2: Degree of severity of anemia based on Hgb value at Jimma medical center, southwest Ethiopia, October 2022

Degree of severity	Frequency	Percent
Mild	47	18.8%
Moderate	92	36.8%
Severe	111	44.4%
Total	250	100%

5.3. Morphological typing of anemia based on the manual peripheral blood smear examination

The most common morphological type of anemia revealed by the manual peripheral blood smear examination was microcytic hypochromic anemia 113 (45.2%), followed by normocytic normochromic anemia 107 (42.8%) respectively **(Table 3).**

Table 3: Shows morphological typing of anemia based on the manual peripheral blood smear examination at Jimma medical center, southwest Ethiopia, October 2022

PBS examination	Frequency	Percent
Microcytic hypochromic anemia	113	45.2%
Normocytic normochromic anemia	107	42.8%
Macrocytic normochromic anemia	24	9.6%
Dimorphic anemia	6	2.4%
Total	250	100%

5.4. Morphological typing of anemia based on RBC indices and histograms obtained from an automated hematological analyzer

From the findings of the red cell indices, the predominant morphological typing of anemia found using the red cell indices was normocytic normochromic anemia 120 (48.0%), followed by microcytic hypochromic anemia 105 (42.0%), **(Table 4)**. The histograms obtained from the analyzer predominantly showed a normal curve in about 107 (42.8%) of cases, followed by left shift in 103 (41.2%) of cases, respectively **(Table 5).**

Table 4: Shows the morphological typing of anemia using red cell indices obtained from an automated hematological analyzer at Jimma medical center, southwest Ethiopia, October 2022

Types of anemia based on the red cell indices	**Frequency**	**Percent**
Normocytic normochromic anemia	120	48.0 %
Microcytic hypochromic anemia	105	42.0 %
Macrocytic normochromic anemia	25	10.0 %
Total	250	100 %

Table 5: Shows the histograms patterns obtained from an automated hematological analyzer at Jimma medical center, southwest Ethiopia, October 2022

Histograms patterns	**Frequency**	**Percent**
Normal curve	107	42.8 %
Left Shift	103	41.2 %
Right shift	16	6.4 %
Bimodal	15	6.0 %
Broad	9	3.6 %

5.5. Histograms patterns of the morphologically typed anemia based on red cell indices

From the total of 120 normocytic normochromic anemia found based on the red cell indices, 104 (86.6%) showed a normal curve, and about 11 (9.2%), 2 (2.7%), 2 (2.7%), and 1 (0.8%) showed bimodal, left shift, broad, and right shift histogram patterns, respectively. Out of 105 microcytic hypochromic anemia found from the red cell indices, the majority showed a left shift histogram pattern, which was about 101 (96.2%), followed by 3 (2.9%), and 1 (0.9%), normal curve, and bimodal histogram patterns respectively. From 25 cases of macrocytic normochromic anemia, the majority of the histogram pattern showed a right shift, which was about 15 (60.0%), followed by 7 (28.0%), and 3 (12.0%), broad and bimodal histogram patterns, respectively **(Figure 1).**

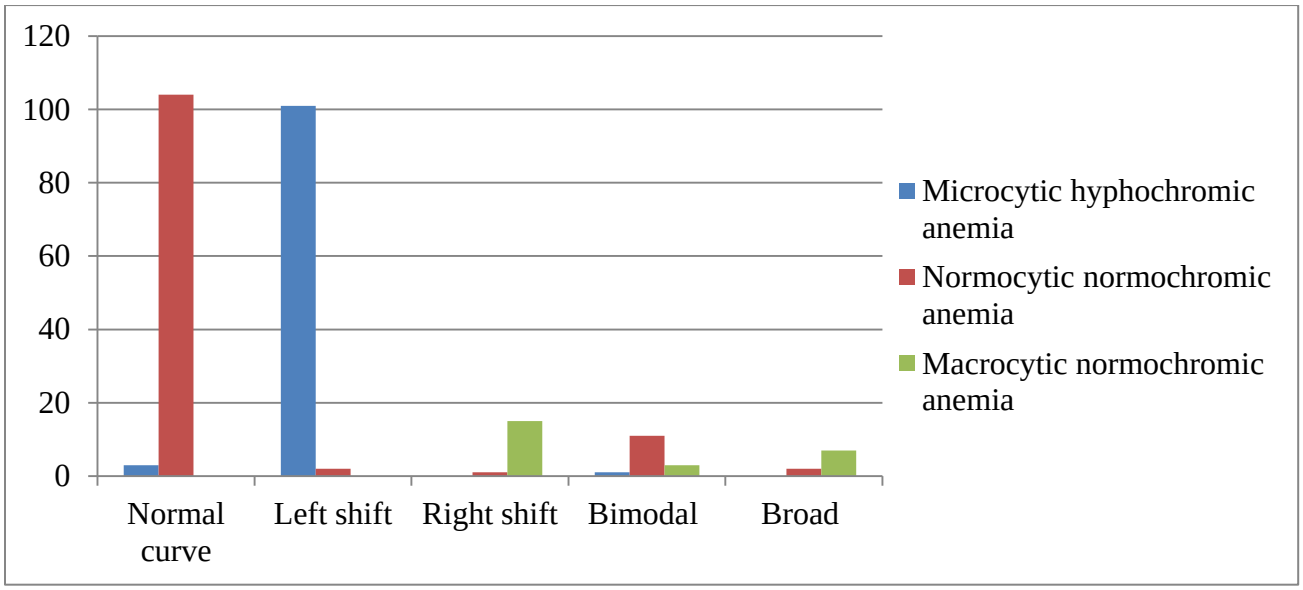

Figure 1: Shows the histograms patterns for the morphologically typed anemia based on red cell indices at Jimma medical center, southwest Ethiopia, October 2022

5.6. Correlation of manual peripheral blood smear examination with red cell indices

Out of the 250 blood samples analyzed, 98 (39.2%) were normocytic normochromic anemia, 97 (38.8%) were microcytic hypochromic anemia, and 22 (8.8%) were macrocytic normochromic anemia with both methods. Cohen's kappa coefficient (k) was 0.8, which shows a strong agreement between the two methods. The concordant and discordant rates between the two methods were 86.8% and 13.2%, respectively. There was a statistically significant difference (p=0.000) between the two methods in the morphological typing of anemia **(Table 6).**

Table 6: Shows the correlation of manual peripheral blood smear examination and red cell indices obtained from an automated hematology analyzer at Jimma medical center, southwest Ethiopia, October 2022

Variables		Red cell indices				P-value
		Normocytic normochromic anemia	Microcytic hypochromic anemia	Macrocytic normochromic anemia	Total	
PBS	Normocytic normochromic anemia	98	7	2	107	P=0.000
	Microcytic hypochromic anemia	16	97	0	113	
	Macrocytic normochromic anemia	2	0	22	24	
	Dimorphic anemia	4	1	1	6	
	Total	120	105	25	250	

5.7. Correlation of the PBS examination with the histogram pattern obtained from the automated hematological analyzer

From the total of 113 microcytic hypochromic anemia found from the peripheral blood smear examination, about 98 (86.7%) showed a left shift, 13 (11.5%) showed a normal curve, 1 (0.9%) showed a bimodal and 1 (0.9%) showed a broad histogram patterns. From 107 cases of normocytic normochromic anemia found from the PBS examination, about 94 (87.8%) showed a normal curve, and about 6 (5.6%), 5 (4.6%), and 2 (1.8%) showed bimodal, left shift, and broad histogram patterns, respectively. From the 24 macrocytic normochromic anemia cases found from the PBS examination, 16 (66.7%) showed a right shift, and 6 (25.0%) and 2 (8.3%) showed broad and bimodal histogram pattern, respectively. All cases of dimorphic anemia found from PBS examination showed bimodal histogram pattern on the automated hematology analyzer.

There was a statistically significant difference (P=0.000) between the PBS examination and the RBC histogram patterns. Cohen's kappa coefficient was k = 0.8, which shows a strong agreement between the histogram patterns and the PBS examination **(Figure 2).**

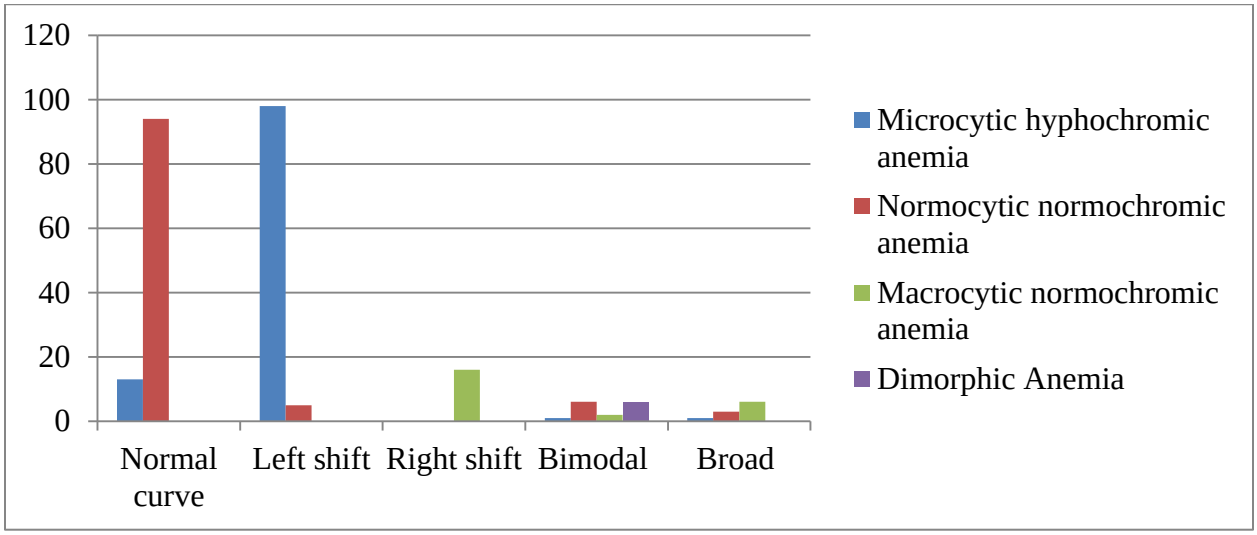

Figure 2: Shows the correlation of morphologically typed anemia by PBS examination with the histogram patterns obtained from the automated hematological analyzer at Jimma medical center, southwest Ethiopia, October 2022

5.8. Sensitivity, Specificity, PPV, and NPV of the red cell indices and histograms obtained from the Beckman coulter UniCel DxH-800 automated analyzers on morphological typing of anemia

The sensitivity, specificity, PPV, and NPV of the red cell indices and histograms obtained from the Beckman Coulter UniCel DxH-800 automated hematological analyzer for morphological typing of anemia were calculated by taking the peripheral blood smear as the gold standard test and comparing the findings of the red cell indices and histograms with the findings of the peripheral blood smear. So, the sensitivity, specificity, PPV, and NPV of the red cell indices and histograms obtained from the automated analyzer were 85.8%, 94.1%, 92.4%, and 88.9% for microcytic hypochromic anemia, 91.6%, 84.6%, 81.7%, and 93.1% for normocytic normochromic anemia and 91.7%, 98.7%, 88.0%, and 99.1% for macrocytic normochromic anemia respectively.

Table 7: Shows the sensitivity, Specificity, PPV, and NPV of RBC indices and histograms obtained from Beckman Coulter UniCel DxH-800 automated hematological analyzer at Jimma medical center, southwest Ethiopia, October 2022

Type of anemia	Sensitivity	Specificity	PPV	NPV
Microcytic normochromic anemia	85.8%	94.1%	92.4%	88.9%
Normocytic normochromic anemia	91.6%	84.6%	81.7%	93.1%
Macrocytic normochromic anemia	91.7%	98.7%	88.0%	99.1%

5.9. Correlation of the RDW obtained from the automated hematological analyzer with anisocytosis in PBS examination

From the total of 250 blood samples analyzed, 175 (70%) showed an increased RDW and about 75 (30%) showed a normal RDW on the automated hematological analyzer. From the findings of the manual peripheral blood smear examination, anisocytosis was seen in about 167 (66.8%) and not seen in 83 (33.2%) cases. Increased RDW and anisocytosis were found in 160 of the cases, and normal RDW with no anisocytosis were found in 68 of the cases. The concordant and discordant rates between RDW and anisocytosis were 91.2% and 8.8% respectively. There was a statistically significant difference ($p = 0.000$) between the RDW and anisocytosis **(Table 8).**

Table 8: Shows the correlation of the RDW obtained from the automated hematological analyzer with anisocytosis in PBS examination at Jimma medical center, southwest Ethiopia, October 2022

Variables		Anisocytosis in PBS			P-value
		Yes	No	Total	
RDW	Increased	160	15	175	P=0.000
	Normal	7	68	75	
	Total	167	83	250	

6. DISCUSSION

Peripheral blood examination has served as a window into hematological developments for decades. The analysis of blood films regularly has aided in the interpretation and diagnosis of many hematological disorders and has served as a key diagnostic tool, particularly in the investigation of anemia (14). Nowadays, the use of an automated hematology analyzer in a hematology laboratory is regarded as a routine as well as an essential requirement (37).

In this study, the findings of the manual peripheral blood smear examination and the red cell indices and histogram patterns obtained from the automated hematology analyzers were compared. According to this study, of the total of 250 anemic samples 58.4% female and 41.6% male samples were analyzed. The major types of anemia based on the Hgb level were severe anemia in 44.4% of cases followed by 36.8% cases of moderate anemia.

An initial morphological classification of anemia that incorporates red blood cell indices and peripheral blood smear examination is likely, to be the most effective (45). Morphologically, anemia can be, classified into microcytic, normocytic, and macrocytic based on RBC size (5). The morphological typing of anemia is also critical for the appropriate treatment of underlying disease (4).

According to the findings of this study, 45.2% of cases had microcytic hypochromic anemia by manual peripheral blood smear examination. Microcytic hypochromic anemia was associated with iron deficiency, pregnancy, menstrual blood loss, and malignancy (46). These findings were comparable with the study conducted by Sandhya V. *et al* (31), Bhatt N *et al* (32), Rao B *et al* (38), Jain A *et al* (33), Singhal S *et al* (8), Jansari T *et al* (34), Choudhary S *et al* (37), and Ashok C *et al* (41) in which microcytic hypochromic anemia was the predominant anemia found with the findings of PBS examination.

The findings differ from a study conducted by Samly D *et al* (36) in which the most common morphological type of anemia was normocytic normochromic anemia in 57.0% of cases. This difference may be due to the difference in the study area; according to Samly D *et al* (36) chronic kidney disease and decreased marrow response were the major causes of normocytic normochromic anemia in India and the sample size difference may be another reason for the

varation, in the study of Samly D *et al* (36) 110 samples were used, which were half of the sample size of this study.

On the other hand, in contrast to the PBS examination, the predominant anemia with the findings of the red cell indices obtained from the automated hematological analyzer was normocytic normochromic anemia, which was in 48.0% of cases. These findings were comparable with the study conducted by Samly D *et al* (36) in which normocytic normochromic anemia was the predominant anemia, followed by microcytic hypochromic anemia, by the automated hematological analyzer. However, the findings of this research were different from a study conducted by Jain A et al (33), Singhal S et al (8), Jansari T et al (34), Garg M et al (39), and Ashok C et al (41) in which microcytic hypochromic anemia was the predominant anemia followed by normocytic normochromic anemia by the result of red cell indices obtained from an automated hematology analyzer.

In this study, the concordance between RBC indices and peripheral smear examination in morphological typing of anemia was 86.8%, and the discordance rate was 13.2%. According to this study, about 13.2% of cases required a peripheral smear review, showing that manual review of blood smears was helpful at least in some cases as it provided additional information for the proper morphological classification of anemia. The benefit of manual screening is its ability to identify clinically important red cell abnormality (pencil cells, tear drop cells, burr cells, schistocytes, target cells, sickle cells, blast cells etc.) that are not detected by the automated analyzers (5).

The discordance can be due to the presence of abnormal red cell shapes which was seen on the PBS examination such as pencil cells, tear drop cells, burr cells, schistocytes, target cells, sickle cells, agglutinated RBCs, fragmented RBCs, spherocytes and so on that were not detected by an automated hematology analyzer. These findings were similar to a study conducted by Singhal S et al (8), and Venukumar M *et al* (35) in which concordant and discordant rates were 88.6% and 11.4%, and 87.75% and 12.25% respectively.

These findings was higher than the concordant and discordant rates reported by Farah E *et al* (5), Choudhary S *et al* (37), and Sandhya V. *et al* (31) studies, which were 78% and 22%, 67% and 33%, and 51.8%, and 48.2%, respectively. This difference may be due to the different analyzers

used; in this study, Beckman Coulter's UniCel DxH-800 automated analyzer was used, whereas in the study conducted by Choudhary S et al. (37) SYSMEX XS-1000i was used. Another factor contributing to the disparity was the difference in sample size; in the study of Choudhary S et al. (37) about 600 anemic patient samples were used, which were three times higher than the sample size of this study.

A peripheral blood smear examination is often used as the gold standard for the diagnosis of numerous RBC, white blood cell, and PLT disorders (47). In this study, the sensitivity, specificity, PPV, and NPV of the red cell indices obtained from the automated hematological analyzer were determined by taking the PBS examination as the gold standard method. According to the findings of this study, the sensitivity, specificity, PPV, and NPV of the red cell indices obtained from automated analyzers were 85.8%, 94.1%, 92.4%, and 88.9% for microcytic hypochromic anemia, 91.6%, 84.6%, 81.7%, and 93.1% for normocytic normochromic anemia, and 91.7%, 98.7%, 88.0%, and 99.1% for macrocytic normochromic anemia, respectively.

The sensitivity of the red cell indices was 85.8%, 91.6%, and 91.7% for microcytic, normocytic, and macrocytic anemia, respectively. This finding was lower than a study done by Jansari T *et al* (34), where the sensitivity of the red cell indices was 94.2%, 96.7%, and 98.2% for microcytic, normocytic, and macrocytic anemia, respectively. According to the findings of this study, the specificity of the red cell indices and histograms was 94.1%, 84.6%, and 98.7% for microcytic, normocytic, and macrocytic anemia, respectively. These findings were comparable with a study done by Venukumar M *et al* (35) in which the specificity of the red cell indices were 91%, 90.5%, and 97.5% for microcytic, normocytic, and macrocytic anemia, respectively.

These findings was higher than a study conducted by Jansari T *et al* (34), Choudhary S *et al* (37) in which the specificity of the red cell indices were 63.1%, 49.1% and 90.3%, and 64.2%, 47.8%, and 98.7% for microcytic, normocytic and macrocytic anemia, respectively. This difference in sensitivity and specificity of the red cell indices and histograms obtained from the automated hematology analyzer may be due to the different analyzers used in different studies. In the study of Jansari T *et al* (40) SYSMEX KX-21 was used, and in the study of Choudhary S *et al* (43) SYSMEX XS-1000i was used. Sample size difference may be the other factor contributes for the

difference; in the study of Jansari T et al. (40) and Choudhary S *et al* (43) 130 and 600 anemic patient samples were used, respectively, in this study 250 anemic patient samples were used.

Red blood cell histograms are graphic representations of blood cells produced from thousands or millions of signals generated by the cells passing through the detector, where they are differentiated by their size and frequency of occurrence in the population. A histogram in association with RBC indices and peripheral blood smears helps in the diagnosis and management of anemia (48). According to the findings of this study, the histograms obtained from the analyzer predominantly showed a normal curve in about 107 (42.8%) cases, followed by a left shift in 103 (41.2%), and about 16 (6.4%), 15 (6.0%), and 9 (3.6%) were right shift, bimodal, and broad histogram patterns, respectively.

The microcytic hypochromic anemia found on the peripheral blood smear examination showed a left-shift histogram pattern in 86.7% of cases. The normocytic normochromic anemia showed a normal curve in 87.8% of cases. All cases of dimorphic anemia showed a bimodal histogram pattern. Seeing these comparison histograms is a useful diagnostic aid in the cases of normocytic normochromic anemia, microcytic hypochromic anemia, and dimorphic anemia. This findings was comparable with the study conducted by Ashok C et al (41). The macrocytic normochromic anemia showed a right shift in 66.7% of cases and broad histogram pattern in 25.0% of cases. Because of the presence of microcytic red cells or small fragmented cells alongside the macrocytic cells, the histogram patterns expanded and form broad base in the case of macrocytic normochromic anemia.

Strength and limitation of the study

Strength

- Peripheral blood smears were reviewed with two trained laboratory technologist.
- Total number of samples for the agreement study was made doubled from that of the minimum CLSI guideline requirement.

Limitation

- Limited studies were available on similar analyzer models, making comparison difficult.

7. CONCLUSION AND RECOMMENDATION

7.1 Conclusion

According to this study, there was a statistically significant difference ($p<0.000$) between a manual PBS examination and the red cell indices and histogram patterns obtained from an automated hematological analyzer. There was a strong agreement ($k = 0.8$) on the morphological typing of anemia between the manual PBS examination and the automated analyzer.

The sensitivity and specificity of the red cell indices and histograms obtained from the automated hematology analyzer showed moderate sensitivity 85.8% and higher specificity 94.1% for microcytic hypochromic anemia, higher sensitivity 91.6% and moderate specificity 84.6 %, for normocytic normochromic anemia and higher sensitivity 91.7% and higher specificity 98.7% for macrocytic normochromic anemia.

Therefore, morphological typing of anemia by incorporating manual peripheral blood smear examination with RBC indices and histograms obtained from an automated hematological analyzer is very useful, provide brief result, and make diagnosis of anemia easier.

7.2. Recommendation

Based on the findings from this study,

- A manual peripheral blood smear examination should be considered as an important laboratory procedure for the investigation of anemia because it provides additional information to the automated hematology analyzer in the diagnosis of anemia.
- Most Clinical Laboratories should practice the use of both methods in diagnosis and morphological typing of anemia in order to make the diagnosis of anemia easier.
- More inspection and quality control should be done on the automated hematological analyzer by comparing to the manual PBS examination in order to generate precise and accurate laboratory result in the diagnosis of anemia.
- Further studies on the comparison between the manual methods and the automated hematological analyzers should be done by including the others hematological parameters like total Rbcs count, total Wbcs count, Hgb, Hct, platelets etc.

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ANNEXES

Annex I: WHO reference range

- WHO (2011) recommendation of hemoglobin (Hb) levels to diagnose anemia at sea level (g/dl) is as follows:
  - Children ½ – 5 years (Hb< 11)
  - Children 5 – 11years (Hb < 11.5)
  - Children 11 – 15years (Hb < 12)
  - Pregnant females (Hb <11)
  - Non-pregnant females (15 years and above) (Hb <12)
  - Men (15 years and above) (Hb<13) (3)

Annex II: SOP for Blood collection

1. Purpose

This SOP describes the procedure for blood collection for hematological analysis.

2. Responsibility

It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

3. Equipment/reagent requirements

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- Blood collection tube: EDTA

4. Procedure

1. Draw blood directly into the evacuated EDTA tube. Filling up the tube to the black mark on the tube label indicates that the correct amount of blood has been drawn.

2. Under-filling or overfilling of the tube can affect laboratory results due to the incorrect blood/additive ratio.

3. The blood collection tube is labeled appropriately with a unique study identification number generated and/or a bar code label generated electronically.

4. Invert the tube 8-10 times to avoid the formation of microclots.

5. Record the time that the sample was taken in the study specific documentation or data management system.

6. Transport to the Haematology laboratory within 4 hours at RT (18-22°C) for processing.

Annex III: SOP for Beckman coulter UniCel DxH800 (Danaher Corporation, United states)

1. Purpose

The UniCel DxH 800 Analyzer is a quantitative, automated hematology analyzer for in vitro diagnostic use in screening patient populations found in clinical laboratories. The UniCel DxH 800 analyzer provides a:

- ❖ Complete Blood Count (CBC)
- ❖ Leukocyte 5 Part Differential (Diff)
- ❖ Reticulocyte (Retic) and Nucleated Red Blood Cell (NRBC) on whole blood
- ❖ Total Nucleated Count (TNC) and Red Cell Count (RBC) on Body Fluids (cerebrospinal, serous and synovial) cells enumeration.

2. Abbreviations

CBC	complete blood count	CSF	Colony-Stimulating Factor
Fl	Femtoliters	JUMC	Jimma University Medical Center
Pg	Pictogram	QC	Quality Control
RT	Room temperature	SRV	Sample Rotor Valve
Ml	Microliter	SAM	Sample Aspirating Module
STM	Specimen Transporting Module	SPM	Specimen Transporting Module
BSV	Blood Supply Valve	AMTC	Air Mixing Temperature Control
MTM	Multi-transducer Module	DV	Distribution Valve
SM	System Manager	PSM	Pneumatic Supply Module
WBC	White Blood Cell count	MPV	Mean Platelet Volume
UWBC	Uncorrected White Blood Cell count	NE	Neutrophil percent
RBC	Red Blood Cell count (for Whole Blood and Body Fluids)	LY	Lymphocyte percent
Hgb	Hemoglobin	MO	Monocyte percent
Hct	Hematocrit	EO	Eosinophil percent
MCV	Mean Corpuscular Volume	BA	Basophil percent
UWBC	Uncorrected White Blood Cell count	NE#	Neutrophil absolute number
RBC	Red Blood Cell count (for Whole	LY#	Lymphocyte absolute number

	Blood and Body Fluids)		
Hgb	Hemoglobin	MO#	Monocyte absolute number
Hct	Hematocrit	EO#	Eosinophil absolute number
MCV	Mean Corpuscular Volume	BA#	Basophil absolute number
MCH	Mean Corpuscular Hemoglobin	NRBC	Nucleated Red Blood Cell percent
MCHC	Mean Corpuscular Hemoglobin Concentration	NRBC#	Nucleated Red Blood Cell absolute number
RDW	Red Cell Distribution Width	RET	Reticulocyte percent

3. Materials

Reagent	**consumable supplies**	
UniCel® DxH 800 Diluent (10L)	Distilled water	container for a bleach solution
UniCel® DxH 800 Cell lyse (5 L)	Ethanol (70%)	container for DI water
UniCel® DxH 800 diff pack (3L)	Alcohol resistant marker	5 to 6% solution of sodium hypochlorite
UniCel® DxH 800 Retic pack (3L)	Plastic dispensing bottles	soft cloth or tissue, lint-free swab or tissue
UniCel® DxH 800 clean (5 L)	Gauze	

3.1 Reagent stability and storage:

 Stable up to expiry date and, up to 60 days after opening and store at room temperature except for cleaner it‘s stable up to 90 days after opening.

4. Equipment

✓ UniCel® UniCel DxH 800 haematology Analyzer

- ✓ Printer
- ✓ Test tube cassette
- ✓ LIS computer
- ✓ Barcode reader

5. Sample and container type

5.1 Whole Blood

Collect 3 or 4ml whole blood in K2 or K3 EDTA according to tube manufacturer‘s instructions and procedures in:

- CLSI publication H4-A5 (for capillary)
- CLSI publication H3-A6 (for venipuncture)

Sample volume is 165µL of whole blood in the closed-vial mode or the single tube presentation mode. The minimum sample volume per tube in the closed-vial mode is 1-ml with the proper proportion of blood to anticoagulant.

5.2 Body Fluids

To reduce body fluid sample viscosity, use hyaluronidase to treat synovial fluids prior to analysis according to your laboratory standards. Add in the ratio of 1 mL of synovial fluid to 5 mg of hyaluronidase. Mix for 5 minutes.

5.3 Sample Retention

Store patient sample at RT and do not analyze after 8 hours of collection.

6. Special Safety precautions

Read all product safety data sheet and don‘t attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer‘s recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter representative.

Alerts for Warning and Caution

WARNING indicates a potentially hazardous situation, which, if not avoided, could result in death or serious injury. It may be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis.

7. Calibration

- The UniCel DxH 800 haematology Analyser is calibrated **biannually** by the service engineer or application specialist.
- The calibration procedure consists of comparing instrument measurements to known values for WBC, RBC, HGB, MCV, PLT, and MPV.
- Calibration assures that an instrument's data output accurately reflects sample input.
- Calibration is performed using materials based on or traceable to known reference preparations or materials.
- In general, the procedure may indicate that the instrument requires standardization, by first determining the deviation from 'calibrator reference', and then applying recommended correction factors (CAL factors).
- Beckman Coulter recommends COULTER S-CAL calibrator, or an exact equivalent, as an acceptable alternative to whole blood calibration.
- For best performance, verify and calibrate all the CBC parameters.
- The WBC differential, NRBC and Retic parameters are calibrated by an authorized Beckman Coulter Representative in your laboratory. The VCSn parameters do not require calibration in the laboratory.

NOTE: Ensure your SPM is properly maintained and the apertures are clean prior to calibration.

8. Quality control

❖ 3 level commercial control which is called **Coulter 6C cell control**, **Coulter Retic-X cell control**, and **Coulter Body fluid control** are used as a quality control material

❖ Whenever the commercial control is stock out validated in-house control is used as a replacement.

❖ The quality control is run every working morning and whenever it is needed i.e.

- If the instrument is calibrated
- After major instrument service maintenance.
- When a new lot of reagent opened.

9. Procedure

9.1 Run Samples

Cassette Presentation

1. Ensure the SPM is set up for the appropriate test for your workflow.

2. Ensure your specimens have been collected and stored properly.

3. Load the specimens into the cassettes.

4. Place the cassettes into the input buffer to the right of the SPM. The SPM automatically begins cycling the cassettes.

5. After the SPM cycles the samples, review the sample results at the System Manager.

Single-tube Presentation

1. Ensure your specimens have been collected and stored properly.

2. Select the Single-tube Presentation icon at the top of any screen to display the Single-tube Presentation dialog box.

3. Place the specimen on the bar-code reader platform of the Single-tube Presentation Station with the bar code facing the SPM to allow the Single-Tube Presentation Bar-code Reader to scan the specimen label, if no barcode please write patient ID manually.

4. Verify the **Specimen Identifier** and **Test** request. Acknowledging the ID that displays on the System Manager screen indicates that you accept the bar-code label read or manual entry.

5. Mix the specimen according to your laboratory standards.

6. Place the specimen into the correct Single-tube position.

Clearing an Exception from the Not Processed Tab

1. From the Work list - Not Processed tab, select or the Exceptions you want to clear.

2. Select the **Clear** button to display the Clear Exceptions dialog box.

3. Select from the following options:

- ❖ Selected Exceptions
- ❖ All Exceptions in Current Filter

4. Select **OK** to clear the selected exceptions.

How to Review Patient Results

To access the Patient Results screen, do one of the following:

- ❖ Select **Menu > Patient Results**.
- ❖ Select a result, and then select the **Details** button on the Work list screen.
- ❖ **Tap** a result twice on the Work list screen.

- ❖ Results are highlighted with a yellow background if action limits are exceeded and results are highlighted with a red background if critical limits are exceeded.
- ❖ Flags are contained in a column next to the results.
- ❖ Non-numeric codes replace results.
- ❖ The Panels Tab is the default tab when there is only one Run Order for a patient's results.
- ❖ A History tab will be available on the Patient Results screen if there are one or more released specimens associated with the patient.
- ❖ A Rerun tab will display if a Rerun has been done.

Release Results

Patient Results can be released from the Panels Tab or the Rerun Tab (for the selected) when the "Release" button is selected. There is no Review Tab for the Patient Results screen.

1. Select the **Release** button on the Patient Results - Review tab to release the results.

2. Select **Yes** to release the panels,

Reject Results

Patient Results can be rejected from the Panels tab or the Rerun tab (for the selected) when the **Reject** button is selected.

1. Select the **Reject** button on the Patient Results screen to reject the results. A UniCel DxH dialog box displays the following message: **Are you sure you want to reject these selected results?**

10. Result interpretation

A low haemoglobin level indicates anaemia. However, haemoglobin findings are even more dependent upon the total number of RBC's. In other words, for the diagnosis of anaemia, the

number of RBC's is as important as the haemoglobin level. In response to an acute infection, trauma, or inflammation, white blood cells release a substance called colony-stimulating factor (CSF).CSF stimulates the bone marrow to increase white blood cell production.

11. Whole Blood Reference range

Parameter	Units	Overall		
		Mean	95% Confidence Low Limit	95%Confidence High Limit
WBC	x103/µl	6.3	3.6	11.2
RBC	x106/µl	4.52	3.73	5.5
HGB	g/dl	13.4	11.4	15.9
HCT	%	39	33.3	45.7
MCV	fL	86.4	73.7	95.5
MCH	Pg	29.6	24.3	33.2
MCHC	g/dl	34.2	32.5	35.8
RDW	%	13.8	12.3	17
RDW-SD	fL	41.4	37.1	47.8
PLT	x103/µl	257	159	386
MPV	fL	9.2	7.5	11.2
NE	%	58.5	43.3	76.6
LY	%	29.6	16	43.5
MO	%	8.3	4.5	12.5

EO	%	2.8	0.6	7.9
BA	%	0.7	0.2	1.4
NE#	x103/µl	3.7	1.8	7.8
LY#	x103/µl	1.8	1	3.0
MO#	x103/µl	0.5	0.3	1.0
EO#	x103/µl	0.2	0.0	0.5
BA#	x103/µl	0	0.0	0.1
NRBC	/100 WBC	0.1	0.0	0.4
NRBC#	x103/µl	0.01	0.00	0.02
RET	%	1.1	0.5	2.17
RET#	x106/µl	0.0498	0.0221	0.0963
MRV	fL	108.8	97.4	120.2
IRF	—	0.4	0.29	0.53

12. Limitations

All Specimens Misleading results can occur if the specimen is not:

- ❖ properly collected, Stored or transported.
- ❖ Contain clots.
- ❖ Is not properly mixed.

13. Principle

The Beckman Coulter UniCel DxH 800 is a Hematology analyser incorporating new electronic and mechanical design with advanced algorithm technology to perform CBC, white blood cell (WBC) differential, nucleated red blood cell (NRBC), and reticulocyte analysis.

Coulter Method (impedance) accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluents) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

VCS Technology

The COULTER VCS established WBC differential technology using three measurements: individual cell volume, high-frequency conductivity, and laser-light scatter.

The combination of low-frequency current, high-frequency current and light-scattering technology provided abundant cell-by-cell information that is translated by the SPM into data plots.

 Volume analysis

Electronic Leukocyte Volume Analysis using low-frequency current has been used since 1967.Ithas been evaluated as a possible adjunct to the differential white cell count.

 Conductivity Analysis

Cell walls act as conductors to high-frequency current. The current, while passing through the cell walls and through each cell interior, detects differences in the insulating properties of the cell

components. The current characterizes the nuclear and granular constituents and the chemical composition of the cell interior.

 Light Scatter Analysis

Coulter‘s experience in flow cytometer dates back decades to Fulwyler‘s pioneering use of light scatter for cell analysis. Loken et al. and Jovin et al. discuss the relationship of particle size and refractivity to the angle of light scattered from a laser beam.

Reticulocyte Analysis

Reticulocytes are immature, non-nucleated erythrocytes retaining a small network of basophilic organelles, consisting of RNA and protoporphyrin. The enumeration of reticulocytes provides a simple, effective means to determine red cell production and regeneration. The most common means of measuring reticulocytes is to use supra vital dyes, such as New Methylene Blue or Brilliant Cresyl Blue. These dyes precipitate and aggregate the basophilic substances within the reticulocyte, resulting in a granular, staining pattern easily seen with light microscopy. Reticulocyte immaturity is related to cell volume and light scatter. Since more immature reticulocytes are larger, contain more RNA and cause for increased light scatter, the cell volume and light scatter will increase with the immaturity of the cell.

14. Clinical utility

A complete blood count (CBC) gives important information about the kinds and numbers of cells in the blood, especially red blood cells, white blood cells, and platelets. A CBC helps to check any symptoms, such as weakness, fatigue, or bruising. It also helps to diagnose conditions, such as anemia, infection, and many other disorders. In general, the complete blood count can be done as part of routine health examination and general screening.

15. Supporting documents

S.No.	Document Title	Document No.

1	UnicellUniCel DxH 800 Preventive maintenance log sheet	
2	JUMCL Safety manual	

16. Reference

1. Instructions for Use UniCel UniCel DxH 800 Coulter Cellular Analysis System: Hematology Specimen Processing Module with System Manager. (March 2009).

2. Sapphire AC, UniCel DxH BC, Advia S, Xe- S, Bruegel M, Nagel D, et al. Comparison of five automated hematology analyzers in a university hospital setting : Abbott Cell-Dyn Sapphire , Beckman Coulter UniCel DxH 800 , Siemens Advia 2120i , Sysmex XE-5000 , and Sysmex XN-2000. Clin Chem Lab Med. 2015; 53(7):1057–71.

3. Hedley BD, Keeney M, Brown W. Initial performance evaluation of the UniCel UniCel DxH 800 Coulter cellular analysis system. Int J Lab Hematol. 2011; 33(1):45–56.

4. Tan BT, Nava AJ, George TI. Evaluation of the Beckman Coulter UniCel UniCel DxH 800 and Abbott Diagnostics Cell-Dyn Sapphire Hematology Analyzers on Pediatric and Neonatal Specimens in a Tertiary Care Hospital. Am J Clin Pathol. 2011; 135(6):929–38.

Source: Jimma University Institute of Health, Jimma Medical Center Laboratory SOP for CBC analysis using Beckman coulter UniCel DxH 800 automated hematological analyzer, Jan 2020.

Annex IV: SOP for manual peripheral blood smear examination

1. Purpose

The purpose of this document is to describe the procedure for preparing manual peripheral blood smears, for the purpose of morphological examination of cells in different haematological disorders..

2. Scope

This procedure is intended for use in clinical trials where patients are attending a clinic for morphological examination. Training of staff is required in order to successfully use the protocol.

3. Abbreviations

mL	millilitres
g	grams
µL	microlitres

4. Duties and Responsibilities

4.1 General information

This section outlines the list of tasks required to complete this procedure. These tasks should be assigned to individual(s) capable of their execution and their name entered beside the task listed in the table below.

4.2 Tasks

	Study personnel
Label slides	
Prepare buffer solutions	

Take blood and make a blood smear	
Stain smear	
Mount slides	

5. Materials and Equipment

- ❖ Two glass slides
- ❖ Pencil
- ❖ Methanol
- ❖ Wright stain
- ❖ Phosphate Buffer PH 6.5.
- ❖ Distilled water

6. Procedure

6.1 Labeling Slides

Glass slides should be clean, grease and scratch free and have smooth edges without any cuts. Label the slides appropriately with a pencil. Write neatly and firmly so that the information can be easily read.

6.2 Making Smears

I. For preparation of a thin smear, a smaller drop (2 to 3µl) of blood should be placed at the end of the slide. Using another slide, the blood can be spread to create a feathered edge that reaches the other end of the slide.

II. The smears must be allowed to air dry free from flies and dust.

III. The smear can be fixed by submerging in methanol for 30 seconds and then letting the slide air dry.

IV. Since methanol fixation would prevent haemolysis, and preserve the morphology of cells.

6.3 Staining of Smears

V. After fixing the slide with methanol, allow to dry for 1-2 minutes.

VI. Stain smears with wright stain for 1-2 minutes.

VII. Gently add buffer of the same quantity as the stain, and mix by blowing gently on the surface.

VIII. Leave for 3-5 minutes.

IX. Rinse slide carefully with distilled water.

X. Allow slide to completely dry (time will vary depending on ambient temperature, but average is 15 minutes).

XI. Then finally observe the morphological characteristics of the cell under the 100X oil immersion magnification.

6.4 Quality Control

The accuracy of results for the morphological examination of peripheral blood smear is highly dependent on the quality of the preparation of the smears. Care should always be taken to use clean, new slides and to follow the instructions outlined above.

7. References

Required document for Laboratory Accreditation by the College of American Pathologists (CAP), Centers for Medicaid and Medicare Services (CMS) and/or COLA

Grading of Morphological Features

Red Cell Morphology:

RBC morphology characteristics are measured and reported by scanning 10 fields in different areas of the slide with evenly dispersed red cells using the 100X oil immersion objective. This

area is usually where cells are just touching or beginning to overlap. The test code ―RBC Morphology‖ is reported as Normal or Present. The individual components of morphology are reported by using the following grading steps. Variance of one grading step is acceptable tech to tech variation when grading RBC morphology.

1. Uniform Grading System for common RBC abnormalities based on scanning at least 10, 100X oil immersion fields:

   Normal = 0-4 cells/100X field

   1+ = 5-14 cells/100X field, slight but significant increase from normal

   2+ = 15-30 cells/100X field, moderate increase

   3+ = greater than 30 cells/100X field, marked increase

   Morphology included in this grading range: Micro, Macro, Echino, Ovalo, Ellipto, and Stomatocytes.

2. Exceptions for the more uncommon RBC abnormalities:

   1+ = 0-2 cells/100X field

   2+ = 3-5 cells/100X field

   3+ = greater than 5 cells/100X field

   Morphology included in this grading range: Hypo, Poly, Acantho, Schisto, Sickle, Sphero, Teardrop and Target cells.

3. Anisocytosis - Variation in size of RBC -anisocytosis is not reported; the specific cell types of microcytes and macrocytes will be quantitated as 1+, 2+ or 3+ using the grading guidelines listed above.

   Normal RBC size = 7 u +/- 1 μm

   Microcyte = < 6.0 μm

   Macrocyte = > 8.0 μm

a. Macrocytosis is graded based on the number of cells/field that are greater than 8.0 μm. Microcytosis is graded based on the number of cells/field that are less than 6.0 μm.

b. If you have trouble approximating the size of the RBC, use the micrometer eyepiece.

c. After you have committed yourself to the degree of micro and/or macro, check the MCV for correlation.

4. Poikilocytosis- Variation in shape of the RBC –poikilocytosis is not reported; the specific cell types will be quantitated as 1+, 2+ or 3+ using the grading guidelines listed above.

5. Polychromatophilia- Significant amount of immature red cells

a. Polychromatic cells display various shades of gray-blue staining red cells indicating presence of basophilic ribonucleoprotein material

b. Grade the number of cells, not the degree of basophilia, since this varies with technique used in staining.

1+ = 0 - 2 cells/100X field

2+ = 3 - 5 cells/100X field

3+ = greater than 5 cells/100X field

6. Hypochromia- Decrease in hgb content of RBCs

a. Hypochromic cells are abnormally pale in color, that is, have an increased central pallor.

b. When grading, consider both the degree of pallor and the number of cells involved.

c. After you are committed to the degree of hypochromia, check the MCH and MCHC. An MCHC less than 30.0 should have hypochromic cells present. If

there is a discrepancy, get another opinion or leave the smear for further investigation. Be careful about cells with artifacts, which would have sharp central borders.

d. Use the same grading criteria as polychromatophilia**.**

e. Water artefact can be confused with hypochromia and appears as a refractile ring around the central pallor.

7. Other Cellular Abnormalities - The following are not quantitated**:**

a. WBC anomolies resulted as Present:Dohle Bodies, Toxic Granulation, Auer Rods, Vacuolated, Hypersegmented (6 or more segments present),Bilobed, and Agranular Neutrophils.

b. RBC anomolies resulted as Present: Rouleaux, Basophilic Stippling, Pappenheimer Bodies, Howell-Jolly Bodies, and Cabot Rings.

i. Basophillic Stippling should only be called when coarse basophilic stippling is present or when there are 2 or more cells with fine stippling present per high power field.

c. PLT morphology: resulted as Normal, Giant, and/or Agranular, and unable to report.

i. Giant platelets should only be called when the platelets are larger than a normal red cell (larger than 8 μm).

ii. The morphology of ―Unable to report‖ should only be used in very rare cases where not a single platelet is visible upon slide review.

Source: ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features.

Annex V: Result reporting form for PBS

Result reporting format for peripheral blood smear

Age_____ Sex _____ Clinical Diagnosis_____

Id No_____

Red cell size

_____

_____

Red cell color

_____

_____

Red cell shape

_____

_____

Red cell inclusions

_____

_____

Presence of immature cells (i.e. blast stage, reticulocyte etc.)

_____

_____

Possible conclusion

_____

Annex VI: Declaration Sheet

Declaration: -

The undersigned declares that this thesis paper complies with the university‘s regulations and meets the accepted standards concerning originality and quality. Principal investigator also agrees to take responsibility for the research paper project's scientific, ethical, and technical conduct and the provision of required progress reports.

MSc. candidate: - Woyesa Beyene Signature: _____ Date: _____

Approval of the first Advisor

Dr. Tilahun Yemane Signature: _____ Date: _____

Approval of the second Advisor

Mr. Edosa Tadasa Signature _____ Date: _____

Examiner

1. Girum Tesfaye Signature _____ Date: _____

2. Signature _____ Date: _____

Head of the School Signature Date

_____ _____ _____

Place: Jimma, Ethiopia