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Evaluating Peripheral Blood Smear Testing in Palestine: A Step Towards Quality Assurance and Diagnostic Excellence

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Evaluating Peripheral Blood Smear Testing in Palestine: A Step Towards Quality Assurance and Diagnostic Excellence

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Evaluating Peripheral Blood Smear Testing in Palestine: A Step Towards Quality Assurance and Diagnostic Excellence

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Dedication

To

Everyone who stood by my side and never left

Everyone that believed in me and never gave up on me

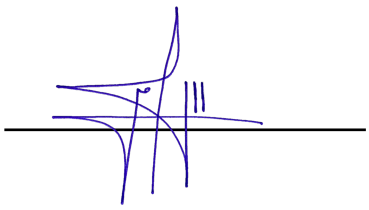
Family, friends, and best friends

I dedicate this work…

Wisam Abdullah Ahmad Alshalash

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed 

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Abstract

Background:

Peripheral blood smear (PBS) test is a critical diagnostic tool in hematology for identifying various blood disorders and malignancies. The absence of a national external quality assurance scheme (EQAS) for PBS testing in Palestine suggests a significant gap in ensuring test quality and standardization. This study aimed to evaluate laboratory technicians' knowledge and practices regarding PBS preparation, staining, and examination, and evaluate laboratory performance regarding PBS examination, and contribute to the establishment of an EQAS program for PBS examination in Palestine.

Methods:

A mixed-methods approach was utilized, consisting of two parts: (1) assessment of laboratory technicians' knowledge and practice about PBS test through a structured questionnaire targeting laboratory technicians across Palestine, with a total of 235 participants, and (2) evaluation of laboratory performance in PBS examination using three pre-stained PBS smears distributed to 39 laboratories that performed both PBS preparation and examination, identified through a preliminary screening survey. Participants were evaluated based on Z-scores for inter-laboratory agreement and the coefficient of variation (CV%) for intra- and inter-laboratory precision.

Results:

Knowledge assessment revealed high performance in smear preparation (92.5%±11.3) but marked deficiencies in staining process (56.7%±21.3), examination methods (60.7%±18.4), and blood cell morphology (64.1%±21.0), with an overall score of 65.9%±14.4. Higher qualifications and active PBS involvement correlated with improved knowledge outcomes. Practical inconsistencies were found in staining process, fixation, drying, and microscope usage. Performance evaluation showed acceptable Z-scores in most parameters (94.3% in WBC counts, 97.1% in lymphocyte differentials, and 100% in platelet counts), yet CV% analysis revealed high intra- and inter-laboratory variability. Morphological agreement with experts was notably low, especially for identifying microcytosis (11.4%), spherocytes (8.6%), and ovalocytes (11.4%).

Conclusion:

The findings highlight significant deficiencies in laboratory technicians' knowledge and substantial variability in PBS-related practices and examination of PBS across clinical laboratories in Palestine. These findings highlight the urgent need for standardized protocols, targeted training, and the establishment of a national EQAS program for PBS examination to enhance diagnostic accuracy and ensure consistent testing quality.

Keywords:

Peripheral blood smear, laboratory performance, quality assessment, external quality assessment scheme, hematology, Palestine, technician knowledge, laboratory practices.

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List of Abbreviations

Abbreviation	Term
EQAS	External quality assessment scheme
PBF	Peripheral blood film
PBS	Peripheral blood smear
CBC	Complete blood count
WBC	White blood cell
EDTA	Ethylene diamide tetraacetic acid
ICSH	International Council for Standardization in Hematology
RBC	Red blood cell
NRBC	Nucleated red blood cell
HPF	High power field
OIF	Oil immersion field
RDW	Red cell distribution width
MCHC	Mean corpuscular hemoglobin concentration
RNA	Ribonucleic acid
HIV	Human immunodeficiency virus
CML	Chronic myelocytic leukemia
PV	Polycythemia vera
CMML	Chronic myelomonocytic leukemia
TPC	Total platelets count
IDA	Iron deficiency anemia
SCA	Sickle cell anemia
HS	Hereditary spherocytosis
HE	Hereditary elliptocytosis
HA	Hemolytic anemia
PBS	Peripheral blood smear
TIBC	Total iron binding capacity
MCV	Mean corpuscular volume
MCH	Mean cell hemoglobin
RES	Reticuloendothelial system
TM	Thalassemia major
TI	Thalassemia intermedia
HPLC	High-performance liquid chromatography
SCD	Sickle cell disease
G6PD	Glucose-6-phosphate dehydrogenase
PKD	Pyruvate kinase deficiency
LDH	Lactate dehydrogenase
MDS	Myelodysplastic syndrome
AMAG	Autoimmune metaplastic atrophic gastritis
ATP	Adenosine triphosphate

CSF	Colony stimulating factor
GM	Granulocyte macrophage
ALL	Acute lymphocytic leukemia
AML	Acute myeloblastic leukemia
CLL	Chronic lymphocytic leukemia
SC	Smudge cell
OMS	Osteomyelosclerosis
ITP	Immune thrombocytopenia
HUS	Hemolytic uremic syndrome
DIC	Disseminated intravascular coagulation
ET	Essential thrombocytosis
BAL	Bronchoalveolar lavage
EQA	External quality assessment
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare & Medicaid Services
DHHS	Department of Health and Human Services
CDC	Centers for Disease Control and Prevention
FDA	Food and Drug Administration
AABB	American Association of Blood Banks
AOA	American Osteopathic Association
ASHI	American Society of Histocompatibility and Immunogenetics
CAP	College of American Pathologists
COLA	Commission on Office Laboratory Accreditation
JCAHO	Joint Commission on Accreditation of Healthcare Organizations
NGO	Nongovernmental organization
SPSS	Statistical package for the social sciences
IRB	Institutional review board
MOH	Ministry of Health
CQML	Center for Quality in Medical Laboratories
PMTA	Palestinian Medical Technology Association
SD	Standard deviation
CV	Coefficient of variation
ISO	International Organization for Standardization
Alg.A	Algorithm A
IQR	Interquartile range
NEQAS	National External Quality Assessment Scheme

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1 Chapter One

Introduction

This chapter presents the research background, outlines the research problem, and explains the justification for the study. It also states the main aim and specific objectives of the research. Finally, the chapter concludes with a summary of the thesis structure.

1.1 Background

A peripheral blood film, or peripheral blood smear (PBS), is a sample of blood that is spread on a glass slide and stained with a special dye (Adewoyin, 2014). Despite the advancements in automated diagnostic techniques in hematology, PBS examination is still a very important screening, diagnostic, and therapy monitoring tool for hematologists in various blood diseases like anemias, leukemias, platelet disorders, and blood parasitic infections (Adewoyin, 2014; Chase et al., 2023; Vu et al., 2021). In cases where a complete blood count (CBC) yields abnormal results, differential morphology recognition of pathological blood cells in a blood smear is a critical point in diagnosis (Gulati et al., 2013; Heller, 1995). The number of cellular components in body fluids increases in several inflammatory disorders, and differential white blood cells (WBCs) are very important. While there are other techniques for identifying

WBCs, manual microscopy is still regarded as the gold standard method (Fleming et al., 2015; Kweon et al., 2022).

Quality control is the main tool enabling laboratories to measure the quality of their results (Sciacovelli et al., 2006). In medical laboratories, PBS examination is a common procedure, and the clinical significance of this analysis and its interpretation are crucial (Vives Corrons et al., 2006). Consequently, monitoring laboratory performance through an external quality assessment scheme (EQAS) is highly recommended (Sciacovelli et al., 2006; Takubo & Tatsumi, 1999; Vives Corrons et al., 2004; Vu et al., 2021). Many studies worldwide have confirmed the role of quality control in improving the quality of medical laboratory results, detecting analytical errors, and taking corrective actions, which in turn leads to better professional performance, better patient outcomes, and better health care service (Fleury et al., 2017; Kristensen & Meijer, 2017; Stavelin et al., 2017). Because of the necessity of quality control and the absence of a national EQAS for monitoring PBS quality, the goal of this study is to assess laboratory technicians' knowledge and practices in PBS preparation, staining and examination, evaluate the performance of laboratories in PBS examination, and identify areas for improvement with the ultimate aim of enhancing the quality of laboratory services and contributing to better healthcare in Palestine.

1.2 Problem statement

Given the importance of PBS examination and its crucial role in the diagnosis and monitoring of many blood diseases and cancer, the examination of PBS is considered one of the basic blood tests in hematology and must be subjected to internal and external quality control processes. The absence of a national EQAS program for monitoring PBS testing quality poses a significant challenge. The lack of a systematic quality control mechanism hinders the ability to ensure consistent and accurate PBS examination results. Therefore, the aim of this study is to reduce this gap by actively participating in the establishment of an EQAS for PBS examination in Palestine.

1.3 Study Justification

The preparation of satisfactory blood smears requires careful attention to the quality of the blood smear and staining techniques, as well as knowledge of the morphologic features of normal and abnormal blood cells. Laboratories measure their working quality through internal and external quality control programs. They have to monitor and thoroughly evaluate every factor that may affect laboratory data, including test results, reference ranges, interpretative comments, and diagnostic workflow. Therefore, monitoring laboratory performance through an EQAS program is highly recommended.

1.4 Research Hypotheses

Because this study is composed of two distinct parts, two corresponding hypotheses are proposed:

Part 1: Technician Knowledge and Practice

- **H0,1**: Laboratory technicians in the West Bank do not exhibit significant deficiencies in knowledge related to the preparation, staining, and examination of PBS tests, their demographic and background characteristics do not influence their knowledge.
- **H1,1**: Laboratory technicians in the West Bank exhibit significant deficiencies in knowledge related to the preparation, staining, and examination of PBS tests, their demographic and background characteristics significantly influence their knowledge.

Part 2: Laboratory Performance

- **H0,2**: There is no statistically significant variability in the performance of medical laboratories in the West Bank in the examination of PBS, as evaluated by intra-laboratory precision and inter-laboratory agreement.

- **H1,2**: There is statistically significant variability in the performance of medical laboratories in the West Bank in the examination of PBS, as evaluated by intra-laboratory precision and inter-laboratory agreement.

1.5 Study Goal

The goal of this study is to contribute to improved healthcare services in Palestine by evaluating and enhancing the quality of PBS testing in medical laboratories. This will be achieved by assessing laboratory technicians' knowledge and practices in PBS preparation, staining, and examination, evaluating laboratory performance in PBS examination, and identifying areas for improvement. The study also aims to support the development of an EQAS as a step toward standardized practices and improved diagnostic accuracy.

1.6 Study Objectives

The specific objectives will include the following:

- Assess the knowledge of laboratory technicians regarding the preparation, staining, and examination of PBS test.
- Evaluate the practices of the laboratory technicians in preparation, staining, and examination of PBS test.
- Prepare and evaluate the homogeneity and suitability of proficiency testing materials for PBS examination.
- Evaluate the performance of participating laboratories in PBS examination, and identify areas of improvement for laboratories with suboptimal performance.

1.7 Expected Outcomes

This study is expected to generate the following outcomes:

- An assessment of the knowledge and practices of laboratory technicians regarding PBS testing.
- Identification of technical and educational gaps that may contribute to suboptimal PBS test quality.
- A comprehensive evaluation of laboratories' performance in PBS interpretation using standardized pre-stained smears.
- Recommendations for implementing an EQAS framework for PBS testing in Palestine.

1.8 Summary of Thesis Chapters

This thesis is organized into five chapters. Chapter One presents an overview of the research problem, the significance of the study, and its objectives. Chapter Two focuses on the literature review relevant to the research problem. Chapter Three outlines the methodology employed in the study. The findings are detailed in Chapter Four, while Chapter Five discusses the results, highlights the main limitations encountered, and offers recommendations based on the study's outcomes.

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2 Chapter Two

Literature review

This chapter provides a comprehensive review of the literature related to PBS testing, beginning with a general overview of PBS preparation, staining, and examination procedures. It highlights the clinical significance of PBS as a diagnostic tool, emphasizing its role in identifying morphological abnormalities associated with various hematological disorders. The chapter also addresses the importance of implementing standardized methods and maintaining quality control throughout the PBS process to ensure diagnostic reliability.

2.1 Blood Smear Overview

The complete blood count and PBS examination are considered the foundation of the laboratory hematologic diagnosis (Ford, 2013). There are several reasons why a blood smear test might be performed. It may be requested by the clinician in response to perceived clinical symptoms or an abnormality in CBC results (Jones, 2009). A microscopic examination of an appropriately prepared and well-stained blood smear by a knowledgeable laboratory professional is still a very important screening, diagnostic, and therapy monitoring tool for hematologists in various blood diseases like anemias, leukemias, platelet disorders, and blood parasitic infections (Adewoyin, 2014; Chase et al., 2023; Gulati et al., 2013; Wintrobe & Greer, 2009). Despite the high level of accuracy and precision, the automated hematology analyzers usually

have data that create a warning flag in 10% to 25% of samples, requiring manual examination of the blood smear; also, many diseases may have normal blood counts but abnormal cellular morphology (Adewoyin, 2014; Chase et al., 2023; Wintrobe & Greer, 2009). A blood smear test can identify abnormal red cell shapes, which can help detect a variety of anemias. A report of abnormal white cell morphology may suggest that extra testing is necessary. Even when the analyzer reports a normal platelets count, abnormal platelet morphology might reveal a platelet function defect (Jones, 2009).

2.2 Blood Smear Preparation

2.2.1 Sample of Choice

Whole blood sample is commonly drawn from peripheral veins and stored in a test tube containing an anticoagulant; the anticoagulant of choice is ethylenediamine tetraacetic acid (EDTA). Because of possible tissue damage and fluid interference, finger pricking is rarely used to get capillary blood. Samples should be delivered to the lab as soon as possible. Blood samples are best tested within two hours of being collected. Delay in preparing a blood smear may cause denaturation of blood cells (Adewoyin, 2014).

According to the International Council for Standardization in Hematology (ICSH), variations in the pH of different EDTA salts, including K_2EDTA, K_3EDTA, and Na_2EDTA, can influence erythrocyte size due to osmotically induced shrinkage. Studies have confirmed that blood cell morphology remains preserved in samples collected using K_2EDTA tubes (Vu et al., 2021).

2.2.2 Smear Preparation

Blood smears can be prepared with either coverslips or glass slides, then usually stained with special dyes (Wintrobe & Greer, 2009).

- Coverslip method Figure (2.1, A): prepared using clean (22 × 22 mm) coverslips, the smear is made by grasping the coverslip by two neighboring corners with the thumb and index finger. A little drop of fresh or

anticoagulated blood is placed in the middle of the coverslip. A second coverslip is then held in a similar manner with the opposite hand, put across the first coverslip, and turned 45° with a steady, fast, and delicate motion. The two coverslips are immediately separated and left to air dry. If done correctly, this process generates two coverslips with uniform distribution of blood and no holes or abnormally thick regions (Wintrobe & Greer, 2009).

- Slides "wedge" method Figure (2.1, B): A drop of blood is placed in the middle of the slide, about 1 to 2 cm from one end. A second spreader slide is positioned at a 30° to 45° angle and moved backwards to make contact with the blood drop. The blood drop spreads along the slide edge, and the spreader slide is quickly dragged ahead. This approach produces a blood film that is 3 to 4 cm long (Wintrobe & Greer, 2009).

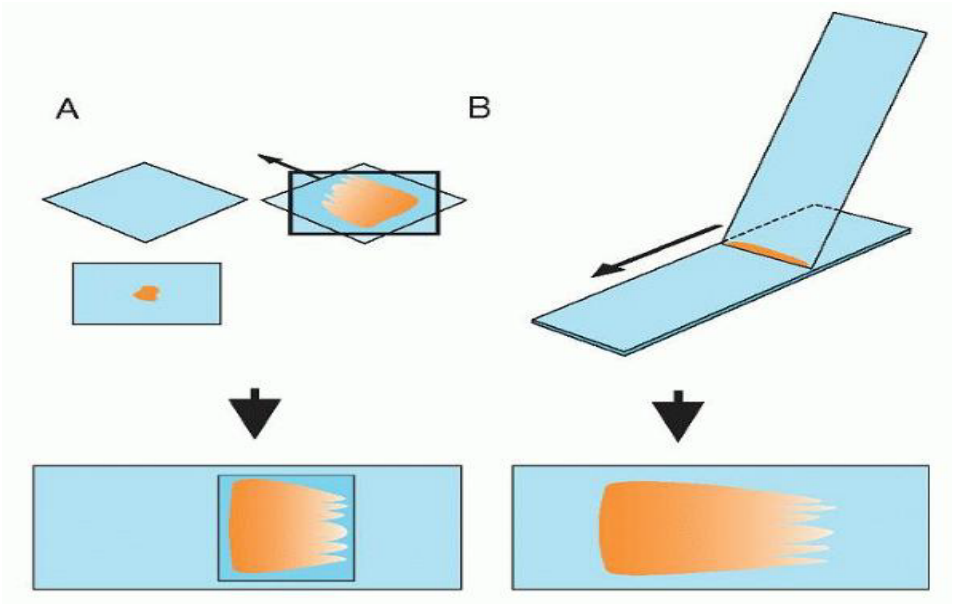

Figure 2.1: Preparation of blood smear. Blood smears may be prepared by the coverslip method (A) or slide wedge method (B) (Wintrobe & Greer, 2009).

2.2.3 Smear Drying and Fixation

Proper air drying of the blood smear is essential to ensure accurate morphological assessment. High humidity should be avoided during smear preparation, as it can lead to inadequate drying, which often results in artefactual sharp refractile borders around the central pallor, making the evaluation of hypochromia challenging. Once dried, the smear is fixed using absolute methanol or ethyl alcohol and subsequently stained with a Romanowsky stain. Ideally, fixation should occur within one hour of preparation but

no later than four hours. Effective fixation requires approximately 10 to 20 minutes, as improper fixation can lead to the formation of artefactual burr cells (Adewoyin, 2014).

Regarding the effect of fixative agents, both pure anhydrous ethanol and methanol effectively preserved the morphology of all blood cell types, maintaining cell integrity for up to 18 hours post-fixation. However, the use of 90% ethanol resulted in water artifacts in erythrocytes. Given the toxicity associated with methanol, ethanol is considered a safer and more suitable fixative agent (Vu et al., 2021). The effect of various preparation conditions and materials is shown in Table 2.1.

Table 2.1: Characterization of blood smears under different preparation conditions (source: (Vu et al., 2021)).

Variable		Erythrocytes	Leukocytes	Platelets	Background
Time before fixation (hours)	0	√	√	√	√
	2	√	√	√	√
	4	√	√	√	
	6	√	√	√	Blue gray streak
Fixative agent	90%-Ethanol	Water artifact	√	√	√
	100%-Ethanol	√	√	√	√
	100%-Methanol	√	√	√	√
Time between fixation and staining (hours)	0	√	√	√	√
	6	√	√	√	√
	12	√	√	√	√
	18	√	√	√	√

√: No effect.

2.2.4 Smear Staining

Blood smears are often stained with either the Wright or May-Grünwald Giemsa stains. Both stains are modifications of the Romanowsky method. The basic stain is composed of methylene blue and eosin. Giemsa stains require known amounts of acid bichromate to produce the azure chemicals. The Wright stain formulation uses sodium bicarbonate to transform methylene blue into methylene azure, which stains the cell (Kweon et al., 2022; Marshall et al., 1975; Wintrobe & Greer, 2009).

The intensity of staining is influenced by both the duration of stain contact and its concentration. It is essential to determine the optimal contact time for each newly prepared or procured batch of stain. Generally, the smear is initially covered with

stain for approximately 5–10 minutes, followed by double dilution with buffered water and an additional 5–10 minutes to allow proper stain absorption by the cells. Finally, the slide is thoroughly rinsed under running water to remove excess stain. Regardless of the type of stain used, the manufacturer's instructions must be followed (Adewoyin, 2014).

2.3 Smear Microscopic Examination

The peripheral blood contains three different types of cells, namely white blood cells (WBCs), red blood cells (RBCs), and platelets (Jones, 2009; K et al., 2022), as shown in Figure 2.2.

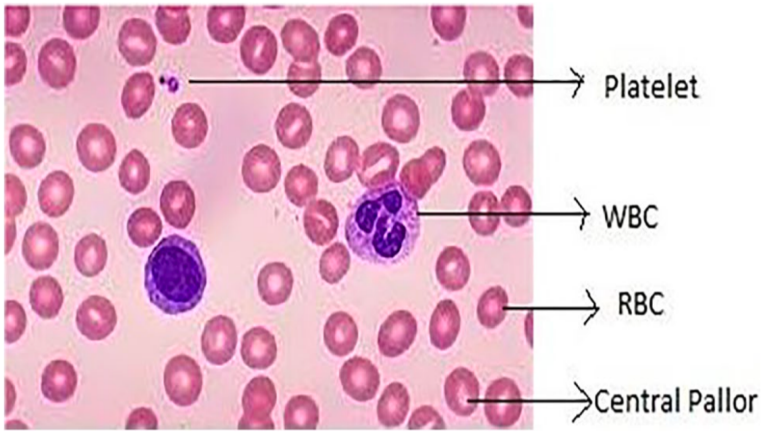

Figure 2.2: Different types of blood cells K et al. (2022).

To determine the overall staining quality of the smear, a low-power objective lens (10x) should be used. It is also used to find the best area for a detailed examination and counting of cells, check the smear for appropriate cell distribution, examine the slide's edges and center for any RBCs, WBCs, or platelet aggregates, search the margins for abnormal cellular components such as blasts forms and nucleated red blood cells (NRBCs), and in some cases, the abnormal cells are located at the edges of the smear, so it is essential to scan over the entire smear to ensure that no cell type is missed (Jones, 2009; Wintrobe & Greer, 2009).

The high-power objective lens (40x), is used to determine the WBCs estimate, correlate the WBCs estimate with the WBC counts from the automated instruments,

evaluate the morphology of the WBCs, and record any abnormalities, such as toxic granulation or Döhle bodies. An estimation of the WBCs count can be done by counting WBCs in ten high-power fields (HPFs), and taking the average, then reporting the count according to the values given in (Table 2.2), or by using a factor, which is based on that each WBC observed in HPF is equivalent to approximately 2000 cells per microliter (µL) of blood (Jones, 2009; Wintrobe & Greer, 2009).

Table 2.2: Estimated WBCs count, average of WBCs in 10 HPFs. (Source: (Jones, 2009))

Average number of WBCs in 10 HPFs	**Estimated total WBCs count / (µL)**
2 – 4	4000 – 7000
4 – 6	7000 – 10,000
6 – 10	10,000 – 13,000
10 – 20	13,000 – 18,000

Oil-immersion objective lens (100x) is used to perform a 100 WBCs differential count (use zig-zag as shown in Figure 2.3), correction of the WBCs count, evaluation of the RBCs for anisocytosis, poikilocytosis, hypochromasia, polychromasia, and inclusions, perform platelets estimate and morphology evaluation (Jones, 2009; Wintrobe & Greer, 2009).

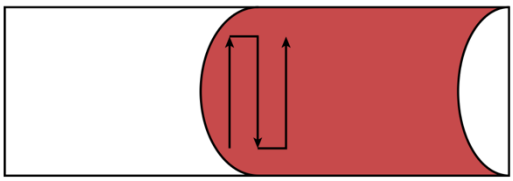

Figure 2.3: A zig-zag manner to perform WBCs differential count (Jones, 2009)

During the WBC differential count, NRBCs should not be included in the total WBC count under any circumstances. If 5–10% NRBCs are observed, they should be reported separately as the number of NRBCs per 100 WBCs. When the number of NRBCs exceeds 10 per 100 WBCs, the total WBC count must be corrected using the following formula (Jones, 2009):

$$\text{Corrected WBCs/mm}^3 = \frac{\text{WBC/mm}^3 \times 100}{100 + \text{No. of NRBCs/100 WBCs}}$$

For platelets estimation, count the average of platelets in 10 oil immersion fields (OIFs), then multiply by 15,000/µL if the smear was prepared by an automated slide spreader, or by 20,000/µL for all manual preparation methods (Jones, 2009; Webb et al., 2004).

2.4 Evaluation of Blood Cells Morphology

Systematic evaluation of the blood smear is essential so that all cell types are examined and characterized. The RBCs' morphology should be evaluated for variations in size (anisocytosis), shape (poikilocytosis), distribution, hemoglobin content, color, and the presence of inclusions. The WBCs morphology examination should include differentiation of white blood cells as well as their general appearance, including nuclear and cytoplasmic abnormalities and the presence of abnormal inclusions that may indicate a disease process. Platelets count should be verified, and the smear should be examined for abnormal platelet shape and size, and for clumping (Jones, 2009).

2.4.1 Red Blood Cells (Erythrocytes)

The normal mature erythrocyte (RBC) has a unique structure in that it lacks a nucleus and organelles yet has all of the components required for life and function (Figure 2.4). It is a biconcave disk with a survival life of around 120 days. On a Romanowsky stain blood smear, this mature red cell appears reddish-orange. RBCs have a diameter of 7–8 µm and an average volume of 90 fL. The center pallor measures around 2 to 3 µm in diameter (Figure 2.4). Figure 2.5 contains a chart of normal and abnormal red cell morphology (Jones, 2009).

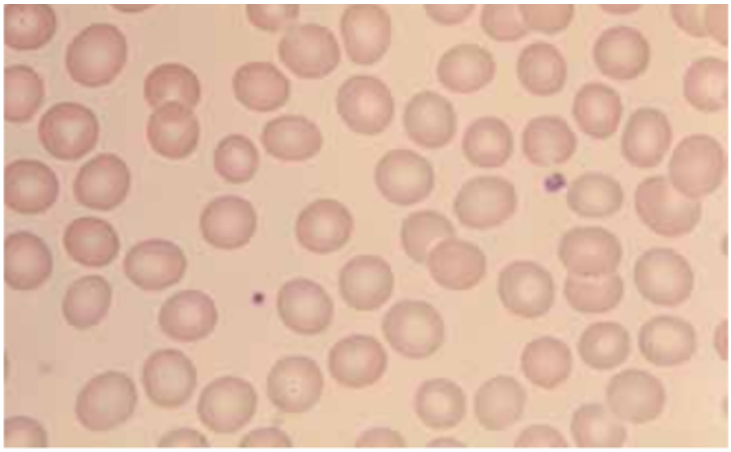

Figure 2.4: Normal red blood cells (Jones, 2009).

The majority of laboratories use either qualitative remarks (*few* or *marked*) or a numerical grading (1 to 4) when reviewing a peripheral blood smear for size and shape variations. The examiner considers the percentage of cells that vary in size in at least 10 OIFs (Ford, 2013; Jones, 2009). See Table 2.2 for an example of grading anisocytosis and poikilocytosis.

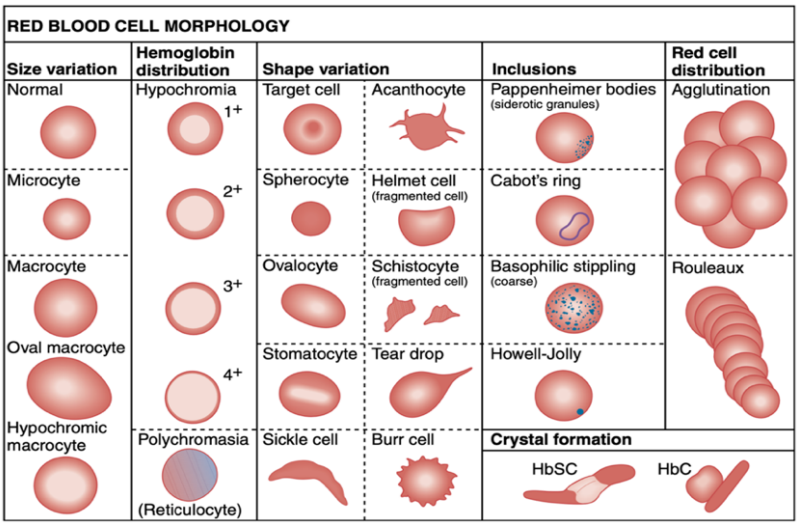

Figure 2.5: Normal and abnormal red blood cells forms (Jones, 2009).

Significant variation in red blood cell size is termed anisocytosis (Figure 2.6). This size variation is commonly observed in leukemia and various forms of anemia. The degree of anisocytosis is typically reflected by an elevated red cell distribution width (RDW). Anisocytosis arises from abnormal cell development, often due to deficiencies in essential materials such as iron, vitamin B12, or folic acid, or from congenital structural defects. Cell sizes can range from smaller than the normal 7 µm to larger than average, with terms like "microcyte" (≤6 µm) and "macrocyte" (≥9 µm)

used to describe these abnormalities. Anisocytosis is usually graded from 1+ to 4+ in most laboratories (refer to Table 2.3 for grading).

Table 2.3: Grading of anisocytosis and poikilocytosis of RBCs. (Source: (Jones, 2009))

Percentage of Cells that Differ in Size or Shape from Normal RBCs	
Normal	5%
Slight	5% - 10%
1+	10% - 25%
2+	25% - 50%
3+	50% - 75%
4+	> 75%

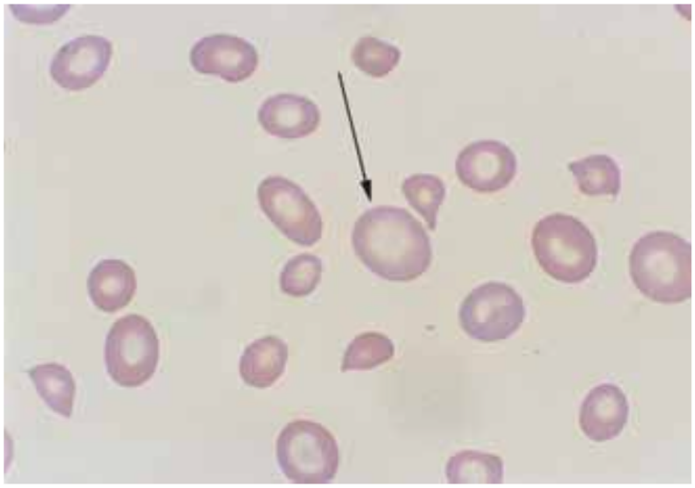

Figure 2.6: Anisocytosis: Size variation between macrocyte (Arrow) and surrounding microcytes (Jones, 2009).

Another important feature to assess in red blood cells is their hemoglobin content. The term "normochromic" refers to red blood cells that appear normal in color, indicating adequate hemoglobin levels. Normochromic erythrocytes have a well-hemoglobinized cytoplasm and a small, defined central pallor area, which should not exceed 3 μm in diameter. In a properly stained peripheral smear, normal red cells display a reddish-orange color. When the central pallor is greater than 3 μm, the cell is considered hypochromic (see Figure 2.7) (Jones, 2009).

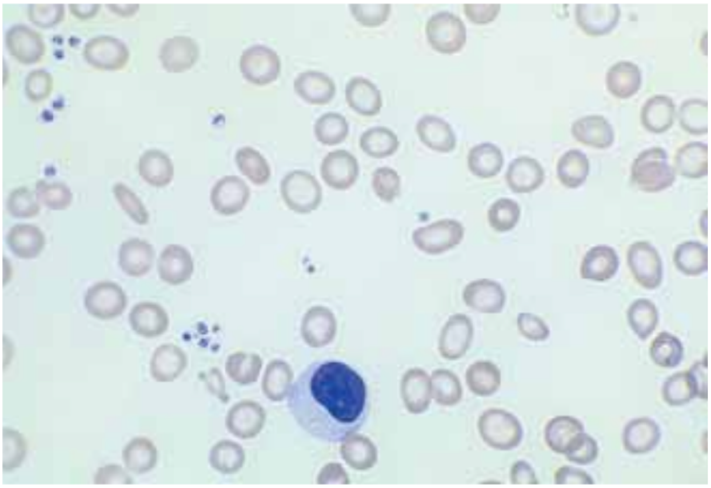

Figure 2.7: Hypochromia: Red blood cells with enlarged central pallor (Jones, 2009).

Many clinicians evaluate hypochromia based on the mean corpuscular hemoglobin concentration (MCHC). When the MCHC is below 32%, the anemia is classified as hypochromic, prompting the slide reviewer to examine the smear for red cells with increased central pallor. A lower MCHC typically corresponds with a larger central pallor in these red cells (Jones, 2009). For guidelines on degree of hypochromia, refer to Table 2.4.

Table 2.4: Guideline for assessing the degree of hypochromia. (Source: (Jones, 2009))

1+	Area of central pallor is one-half of cell diameter
2+	Area of pallor is two-thirds of cell diameter
3+	Area of pallor is three-quarters
4+	Thin rim of hemoglobin

When red blood cells are released into the peripheral circulation prematurely, they have a distinctive appearance on a Wright-stained smear. These cells, known as polychromatophilic cells, appear gray-blue in color and are typically larger than normal red cells (see Figure 2.8). Their basophilic color is due to residual RNA, which is involved in hemoglobin production. Polychromatophilic macrocytes, seen in Wright-stained smears, are actually reticulocytes, though the reticulum itself is only visible with supravital staining (Jones, 2009).

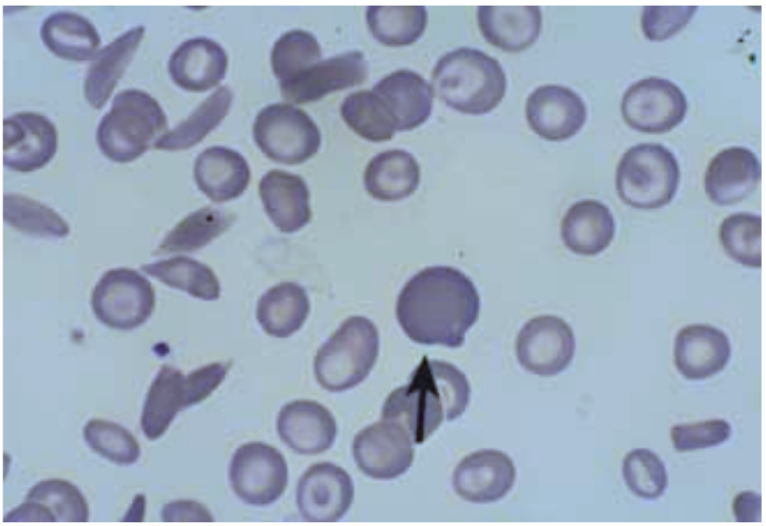

Figure 2.8: Representative cell showing polychromasia (Indicated by Arrow) (Jones, 2009).

It is normal to observe a few polychromatophilic cells in a typical peripheral blood smear, as red cell regeneration is ongoing. The reticulocyte count should correspond to the level of polychromasia observed. In the smear, these cells display varying shades of blue. Any condition that stimulates bone marrow, especially in cases of increased red cell regeneration, will result in a polychromatophilic blood appearance. The degree of polychromasia serves as a valuable measure of treatment effectiveness in patients receiving iron or vitamin therapy for anemia (Jones, 2009). For guidance on grading polychromasia, refer to Table 2.5.

Table 2.5: Guidelines for grading hypochromia. (Source: (Jones, 2009))

Slight	**1+**	**2+**	**3+**	**4+**
1 %	2 %	5 %	10 %	> 11%

Erythrocytes typically exhibit uniform hemoglobin distribution after staining; however, in reticulocytes, the remains of ribosomes appear as substantia granulofilamentosa after supravital staining. Under certain conditions, ribosomal material may aggregate, showing up as basophilic stippling, which is also detectable with Giemsa staining. While this is normal in fetal and infant blood, in adults, a significant diagnostic clue arises when a large number of basophilic stippled erythrocytes are observed alongside anemia, suggesting conditions like thalassemia, lead poisoning, or sideroblastic anemia (Theml & Diem, 2011).

Occasionally, small spheres about 1μm in diameter, representing errant chromosomes from the mitotic spindle, may be found in normoblasts. These structures, known as

Howell-Jolly bodies, are normally eliminated by the spleen but may persist in the erythrocyte after nuclear expulsion, appearing as irregularly distributed inclusions. They are commonly observed after splenectomy and, less frequently, in hemolytic conditions or megaloblastic anemia. Howell-Jolly bodies are visible with standard Romanowsky staining (Theml & Diem, 2011).

Supravital staining may also reveal Heinz bodies and rare globular precipitates at the cell membrane, indicating unstable hemoglobin in certain familial or toxic hemolytic conditions. Additionally, ellipsoid, eosinophilic-violet-stained rings within erythrocytes, called Cabot rings, are thought to be composed of fibers from the mitotic spindle rather than remnants of the nuclear membrane. These rings appear sporadically in severe anemias but do not provide specific diagnostic information, though their presence may raise suspicion of an emerging idiopathic erythropoietic disorder in the absence of other clear causes for the anemia (Theml & Diem, 2011). Figure 2.9 exhibits examples of erythrocyte inclusions.

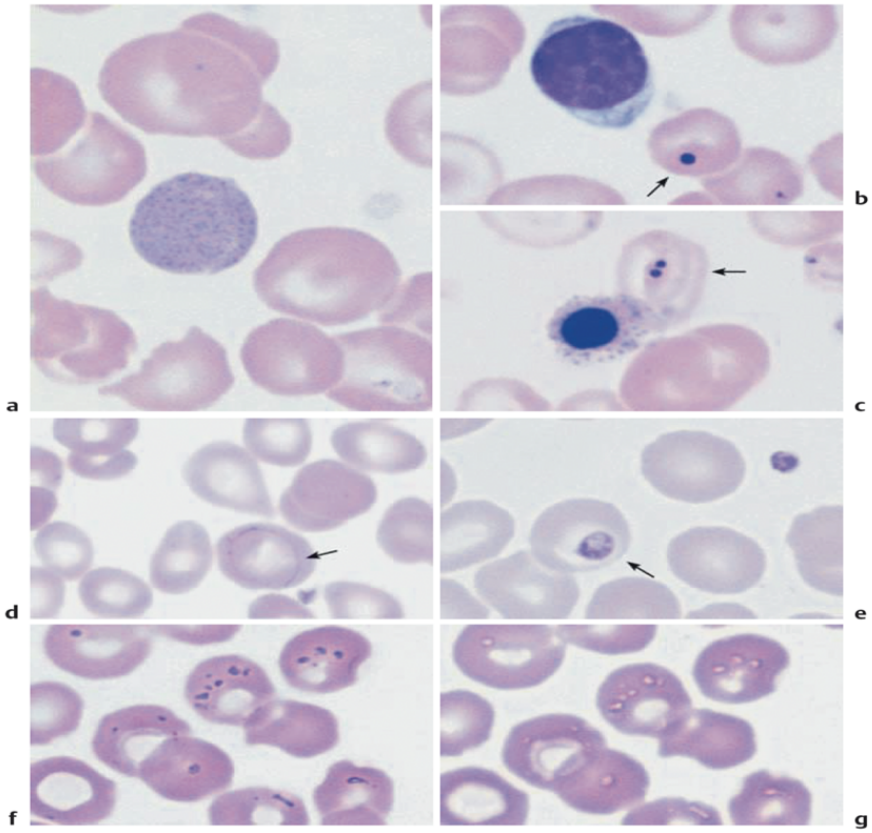

Figure 2.9: Erythrocytes inclusions. **(a)** Polychromatic erythrocyte with fine, dense basophilic stippling. **(b)** Erythrocyte with Howell–Jolly bodies (arrow) in addition to a lymphocyte. **(c)** Erythrocyte with two Howell–Jolly bodies (arrow) alongside an orthochromatic erythroblast with basophilic stippling. **(d)** Erythrocyte with a delicate Cabot ring (arrow). **(e)** Thrombocyte

layered onto an erythrocyte (arrow). **(f and g)** Fixation and staining artifacts (Theml & Diem, 2011).

2.4.2 White Blood Cells (Leukocytes)

Evaluation of WBCs is performed mainly in response to abnormalities identified by the CBC results. There are five different types of WBCs, namely neutrophil, lymphocyte, eosinophil, basophil, and monocyte (Figure 2.10) (Gautam & Bhadauria, 2014). Mature WBCs may undergo various morphological and numerical changes; accordingly, laboratory technologists need to be able to perform a differential count of the WBCs and identify the typical characteristics of normal cells, and the abnormalities in size, shape, abnormal inclusions, immature forms, and overall appearance. While leukocytosis typically denotes an increase in the concentration of peripheral WBC exceeding 10,000 cells/μL, markedly elevated WBC counts exceeding 100,000 cells/μL are more indicative of a myeloproliferative process (Jones, 2009).

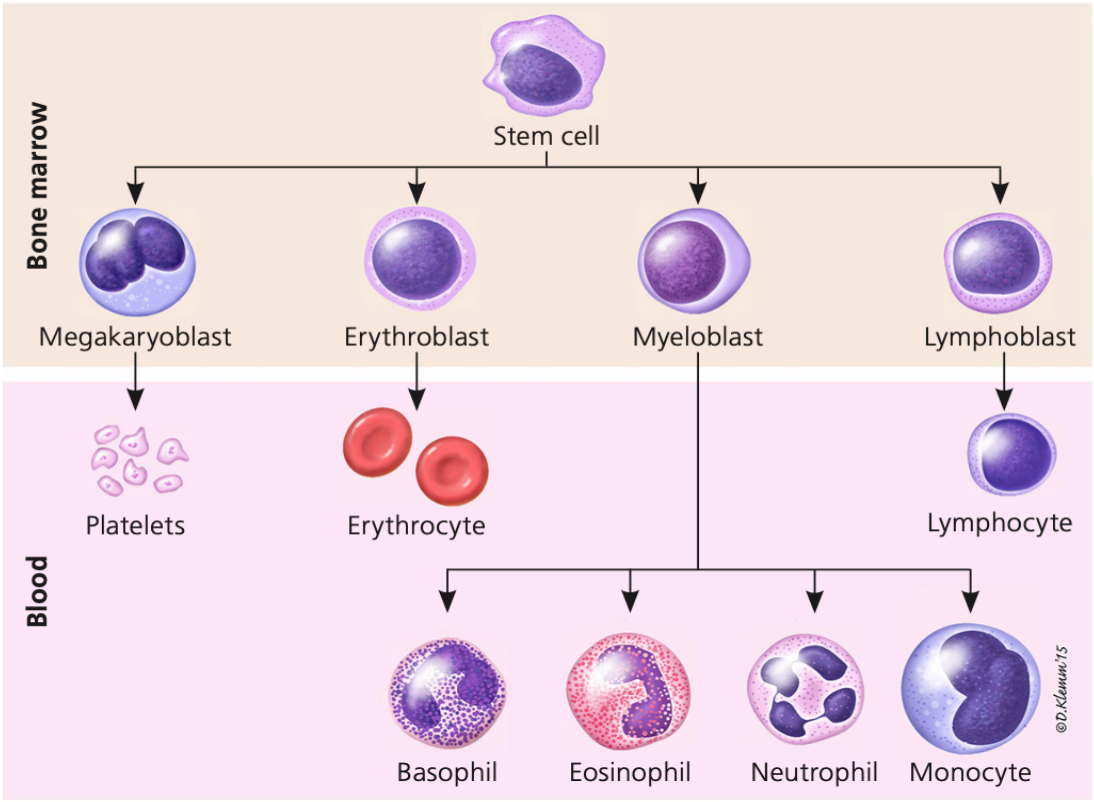

Figure 2.10: White blood cells maturation line (Riley & Rupert, 2015).

2.4.2.1 Neutrophils

Neutrophilia stands out as the most prevalent form of leukocytosis and is historically characterized as the "soldiers" of our innate immune system. Approximately 60% to 70% of mature WBCs in the peripheral blood circulation are neutrophils. Neutrophils demonstrate a wider range of morphologic alterations compared to other cell types, primarily originating in the cytoplasm in response to various pathological processes such as severe infections, inflammatory conditions, or other leukemoid reactions. Features like toxic granulation, toxic vacuolization, or the presence of Dohle bodies may accompany these conditions. An increase in segmentation (normal is two to five lobes) may signal a megaloblastic process, while a decrease in segmentation could indicate a benign hereditary condition called Pelger-Huet anomaly or may be indicative of a leukemic process (Jones, 2009; Tahir & Zahra, 2021).

2.4.2.2 Lymphocytes

Lymphocytes constitute approximately 20 to 40% of WBCs. Lymphocytosis is characterized by an elevation in the absolute lymphocyte count to more than 4000 lymphocytes/μL in adult patients (Hamad & Mangla, 2019). Primary lymphocytosis refers to conditions marked by an increase in the absolute number of lymphocytes due to an intrinsic defect in the expanded lymphocyte population. These conditions, also known as lymphoproliferative disorders, are most commonly linked to the neoplastic accumulation of monoclonal B cells, T cells, natural killer (NK) cells, or less differentiated cells of the lymphoid lineage. Secondary lymphocytosis refers to conditions associated with an increase in the absolute number of lymphocytes due to a physiological or pathophysiological response such as infection, toxins, cytokines, trauma, surgery, acute cardiac failure, septic shock, myocardial infarction, sickle cell crisis, or other unknown reasons (Kipps, 2010).

2.4.2.3 Eosinophils

Eosinophils have diverse roles that include maintaining bone marrow plasma cells, participating in vaccine recall responses, modulating various T cell-mediated responses, contributing to processes like tissue repair, glucose and fat metabolism, and potentially tumor surveillance. The classic definition of eosinophilia involves an elevation to greater than 500 eosinophils/μL, commonly associated with parasitic

diseases such as helminth infections. Furthermore, eosinophilia can be linked to HIV infection; some medications, such as allopurinol, sulfasalazine, antibiotics, and anticonvulsants, may cause persistent eosinophilia. Mild to moderate eosinophilia is associated with autoimmune disorders and various atopic conditions, including atopic dermatitis, allergic rhinitis, and asthma. Additionally, blood eosinophilia may be associated with occult neoplasms (Kuang, 2020).

2.4.2.4 Basophils

An increase in basophil levels can indicate the presence of an underlying neoplasm, such as chronic myeloid leukemia (CML), polycythemia vera (PV), primary myelofibrosis, essential thrombocythemia, acute myeloid leukemia, or, less commonly, solid tumors. More prevalent causes include allergic reactions or persistent inflammation associated with infections (including influenza and tuberculosis), inflammatory bowel disease, and autoimmune disorders. Additionally, drug-related factors and dietary intake are known to be correlated with symptoms and the extent of basophilia (Sticco et al., 2024).

2.4.2.5 Monocytes

As individuals age, there is an expansion in the prevalence of monocytes, which play crucial roles in inflammatory and immune responses. Monocytes comprise 2% to 8% of the overall WBCs count in peripheral blood, ranging from (0.3-0.9) x 10^3/μL. Abnormal production and accumulation of monocytes can occur in various conditions. Monocytosis, an elevated monocyte count, may be attributed to inflammatory conditions referred to as "reactive monocytosis," observed in situations such as acute stress, myocardial infarction, chronic infection, systemic inflammation, and autoimmune disorders. In addition to secondary causes of monocytosis, an increased monocyte count in peripheral blood serves as a characteristic feature of chronic myelomonocytic leukemia (CMML), myelodysplastic syndrome, and myeloproliferative disorders. It mainly affects older individuals. Additionally, monocytosis may coexist with other myeloid malignancies (van Zeventer et al., 2022).

In addition to an increase in various WBC counts, a reduction in the number of circulating WBCs can be observed in different conditions. Leukopenia is characterized by an absolute decrease in the circulating WBCs count below the lower

limit of normal values, encompassing all types of WBCs. However, in medical practice, the term leukopenia is commonly used to refer to neutrophilia. The lowest normal value of neutrophils typically ranges from 1,500 to 1,800/mm, and a decrease in this count may be due to diminished production of white blood cells, increased utilization and destruction, or a combination of both factors. Neutropenia can be caused by factors such as infection, drugs, malignancy, megaloblastosis, hypersplenism, and immunoneutropenia. In children, primary neutropenia may have a hereditary basis and could be associated with other developmental defects (Ing, 1984).

2.4.3 Platelets (Thrombocytes)

The typical platelet (illustrated in Figure 2.11) displays distinct morphological features, measuring approximately 2–4 μm. These cells lack a nucleus and possess cytoplasm filled with granules (Jones, 2009; Umashankar et al., 2014).

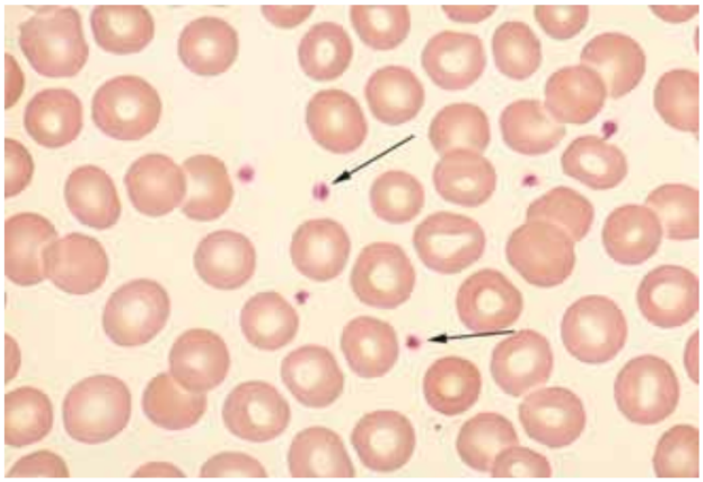

Figure 2.11: Normal platelets (arrows) in peripheral circulation (Jones, 2009).

Under normal circumstances, the platelet count falls within the range of 150 to 450×10^3/μL. However, in various clinical situations, the platelet count may deviate, either increasing (thrombocytosis) or decreasing (thrombocytopenia). It is essential to assess and address both situations. Severe thrombocytopenia, with a platelet count dropping below 20×10^3/μL, manifests as internal and external bleeding and, in some cases, can be life-threatening. Conversely, thrombocytosis may lead to thrombotic events. Accurate platelet count determination is a crucial laboratory parameter for clinical decision-making, such as the administration of platelet transfusions in cases of

severe thrombocytopenia. Consequently, platelet count estimation is a significant and routinely requested laboratory investigation (Umashankar et al., 2014).

The measurement of total platelet count (TPC) is a fundamental component of standard hematology laboratory procedures. Automated cell counters are commonly used by clinical laboratories to perform this task. However, despite technological advancements, the occurrence of falsely low or high platelet counts generated by automated cell counters is a frequent issue in routine laboratory operations. Consequently, most laboratories adopt the practice of verifying any abnormal or questionable platelet values obtained from the automated cell counters by employing an alternative manual estimation method (Sahu et al., 2022). Under the 100x oil immersion, it is expected to observe around 7–15 platelets per oil immersion field (OIF). Each platelet observed per OIF corresponds to roughly 15,000–20,000 circulating platelets (Adewoyin, 2014).

2.5 Clinical Application of PBS

Despite the advancements in automated diagnostic techniques in hematology; PBS examination is still a very important screening, diagnostic, and therapy monitoring tool for hematologists in various blood diseases like anemias, leukemias, platelet disorders, and blood parasitic infections (Adewoyin, 2014; Chase et al., 2023; Vu et al., 2021). In cases where CBC yields abnormal results, differential morphology recognition of pathological blood cells in a blood smear is a critical point in diagnosis (Gulati et al., 2013; Heller, 1995).

2.5.1 Anemia

Anemia is a condition where the number of red blood cells and/or the level of hemoglobin are lower than normal, making them insufficient to satisfy the body's physiological requirements. Anemia is classified based on red cell morphology, indices, and hemoglobin content into hypochromic microcytic, normochromic normocytic, and macrocytic types. It is further categorized into Iron Deficiency Anemia (IDA), Sickle Cell Anemia (SCA), Thalassemia, Hereditary Spherocytosis

(HS), Hereditary Elliptocytosis (HE), Aplastic Anemia, and Hemolytic Anemia (HA) (Chaparro & Suchdev, 2019; KT et al., 2022).

RBC morphology is a key tool for hematologists to recommend appropriate clinical and laboratory follow up and to select the best tests for definitive diagnosis (KT et al., 2022). Figure 2.12 summarizes the classification of the anemias according to red blood cells morphology.

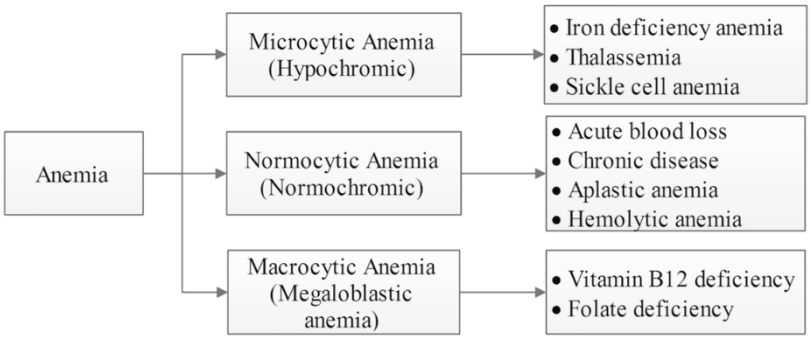

Figure 2.12: Classification of Anemia according to RBC's morphology (KT et al., 2022).

Anemia is diagnosed using a peripheral blood smear (PBS), where microscopic examination of the blood provides valuable insights into changes in RBC shape, size, or the presence of inclusion bodies. During this process, pathologists evaluate the size, shape, and color of RBCs and white blood cells (WBCs), as well as estimate the platelet count. The quality of RBCs is determined by red cell indices, and any abnormalities in size, volume, or shape indicate abnormal red blood cells (Chaparro & Suchdev, 2019; KT et al., 2022).

2.5.1.1 Iron Deficiency Anemia

Iron deficiency anemia (IDA) can arise either from absolute depletion of iron stores or functionally, where iron reserves are present or even elevated, but there is a disruption in the delivery of iron to the bone marrow. These conditions may coexist, leading to disorders in erythropoiesis and hemoglobin synthesis. This type of anemia is characterized by hypochromic microcytic anemia, reduced serum iron and ferritin levels, increased total iron-binding capacity (TIBC), decreased transferrin saturation, negative bone marrow iron staining, and responsiveness to iron treatment (Jain & Kamat, 2009).

The causes of IDA include insufficient iron intake, poor absorption, or chronic iron loss due to prolonged bleeding, leading to an iron-depleted state or negative iron balance (Jain & Kamat, 2009). Mechanisms associated with this condition are illustrated in Figure 2.13.

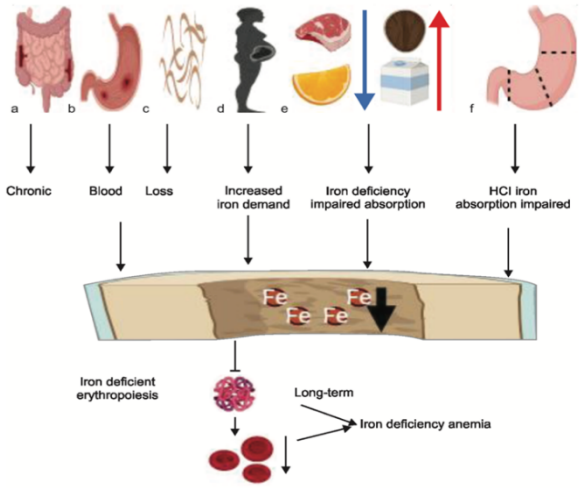

Figure 2.13: Mechanisms of IDA**. (a)** Colonic bleeding; **(b)** Gastric bleeding; **(c)** Hookworm infestation; causing chronic blood loss; **(d)** Increased demand (e.g., pregnancy); **(e)** Low iron intake or diet-related to impaired absorption; **(f)** Gastrectomy; causing decreased HCl and impaired iron absorption (Umar, 2020).

The diagnosis of iron deficiency anemia involves a thorough medical history and physical examination. Common findings include symptoms of anemia, which typically appear when hemoglobin levels drop below 7–8 g/dL (Jain & Kamat, 2009).

Laboratory testing plays a key role in diagnosing iron deficiency anemia. Typical results include decreased mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), serum iron, ferritin, and transferrin saturation. Additionally, there is an increase in total iron-binding capacity (TIBC) and a lack of iron staining (Prussian Blue) in bone marrow analysis, though this test is rarely performed (Jain & Kamat, 2009).

Microcytic hypochromic anemia seen in a peripheral blood smear, examining erythrocyte morphology is the fastest and most effective method for investigating hypochromic anemia when serum iron levels have fallen below normal. In cases of iron deficiency anemia (IDA), the size and shape of erythrocytes usually remain consistent (refer to Figure 2.14). It is only in more severe anemias (around 11 g/dl, or 6.27 mmol/l Hb) that smaller erythrocytes (microcytes) appear, indicating insufficient

hemoglobin content. Reticulocytes can be identified by their resemblance to larger polychromatic erythrocytes (Theml & Diem, 2011).

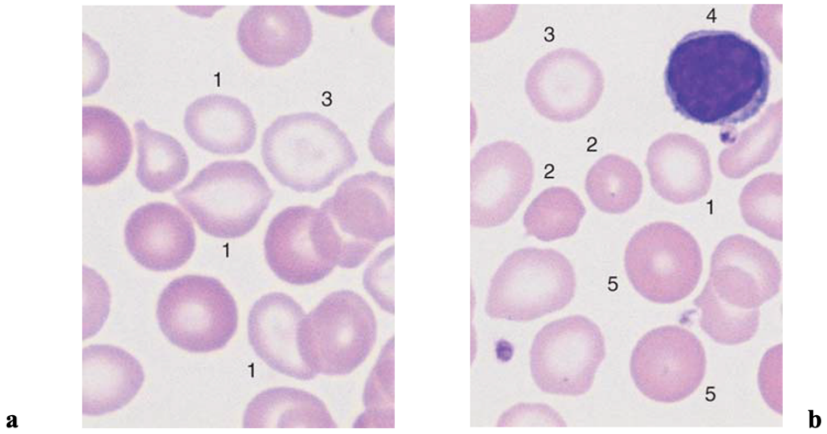

Figure 2.14: Iron deficiency anemia. **(a and b)** Erythrocyte morphology in iron deficiency anemia: ring-shaped erythrocytes (1), microcytes (2) faintly visible target cells (3), and a lymphocyte (4) for size comparison. Normal-sized erythrocytes (5) after transfusion (Theml & Diem, 2011).

Among the various causes of insufficient iron for erythropoiesis, a special case of hypochromic anemia arises from the internal shift of iron due to the "iron pull" by the reticuloendothelial system (RES) during infections, toxic conditions, autoimmune diseases, and tumors. This type of anemia, caused by an underlying disorder, is known as secondary anemia. The mean corpuscular hemoglobin (MCH) is usually hypochromic, though in rare cases it can be normochromic, making erythrocyte morphology particularly significant for diagnosis (Theml & Diem, 2011). See Figure 2.15.

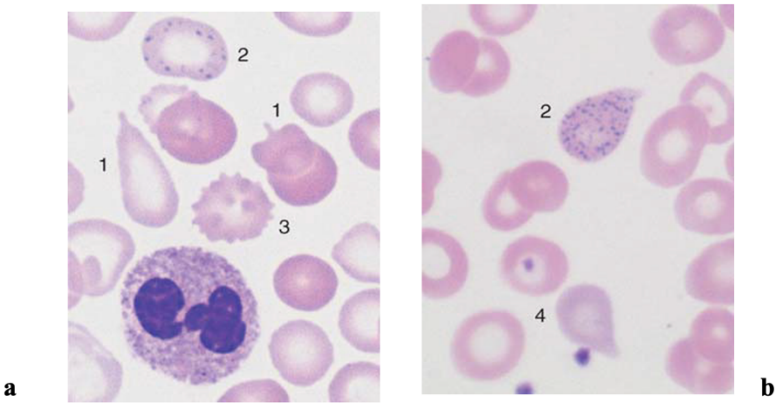

Figure 2.15: Secondary anemia**. (a and b)** Erythrocyte morphology in secondary hypochromic anemia: the erythrocytes vary greatly in size (anisocytosis) and shape (1) (poikilocytosis), and show basophilic stippling (2). Burr cell (3), which has no specific

diagnostic significance. Occasionally, the erythrocytes stain a soft gray–blue (4) (polychromasia) (Theml & Diem, 2011).

2.5.1.2 Sideroblastic Anemia

Sideroblastic anemia is a form of anemia caused by abnormal iron utilization during the production of red blood cells (erythropoiesis); it can be inherited or acquired. It is characterized by the presence of ring sideroblasts in the bone marrow (Figure 2.16), which are erythroid precursors with deposits of non-heme iron in their mitochondria, forming a ring-like pattern around the nucleus. This iron-filled ring covers at least one-third of the nucleus. Sideroblastic anemia can cause either microcytic or macrocytic anemia, depending on the underlying mutation. Unlike iron deficiency anemia, where iron stores are depleted, patients with sideroblastic anemia typically have normal to elevated iron levels (Ashorobi & Chhabra, 2024; Rodriguez-Sevilla et al., 2022).

Ring sideroblasts are erythroblasts that display abnormal accumulation of iron in their perinuclear mitochondria, and their presence is a defining feature of sideroblastic anemias. To detect these ring sideroblasts, a Prussian blue stain (Perls' reaction) must be applied to bone marrow aspirate smears (Rodriguez-Sevilla et al., 2022).

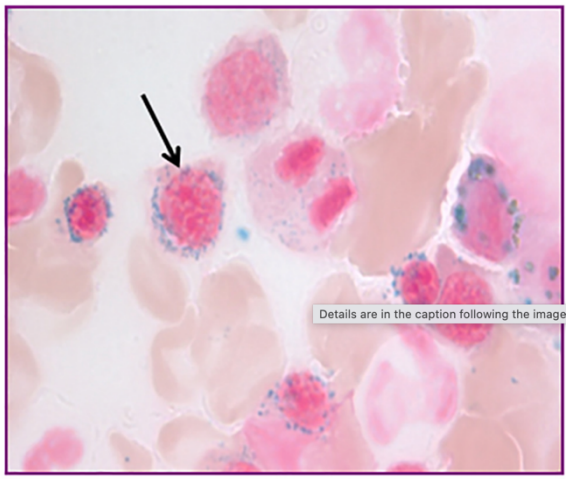

Figure 2.16: Prussian blue stain**.** Bone marrow aspirate stained with Prussian blue stain shows Ring sideroblasts (Rodriguez-Sevilla et al., 2022).

2.5.1.3 Thalassemia

Thalassemia syndromes are a group of inherited blood disorders caused by a reduced or absent synthesis one of the globin chains, leading to lower hemoglobin levels in red

blood cells (RBCs), reduced RBC production, and anemia. Most thalassemias are passed down as recessive genetic traits (Cohen et al., 2004).

Beta-thalassemia (β-thalassemia) is marked by reduced or absent beta globin chain synthesis and consists of three main forms: β-thalassemia major (TM), also known as “Cooley’s anemia” or “Mediterranean anemia”; β-thalassemia intermedia (TI); and thalassemia minor, often referred to as “β-thalassemia carrier” or “β-thalassemia trait" (Langer, 1993; Origa, 2017).

Individuals with β-thalassemia major (TM) typically present between 6 and 24 months of age and require regular red blood cell (RBC) transfusions for survival. Affected infants often fail to thrive, becoming increasingly pale, and may experience feeding difficulties, diarrhea, irritability, frequent fevers, and progressive abdominal enlargement due to splenomegaly. Without regular transfusions, these patients usually die from high-output heart failure (Cohen et al., 2004; Langer, 1993; Origa, 2017). When a regular transfusion regimen is established, maintaining a hemoglobin (Hb) level between 9.0 and 10.5 g/dL, ineffective erythropoiesis is suppressed, and growth and development usually remain normal until around 10 to 12 years of age. However, patients receiving transfusions may face complications from iron overload, which are influenced by their adherence to chelation therapy (Cohen et al., 2004; Langer, 1993; Origa, 2017).

Individuals with β-thalassemia intermedia (TI) present later than those with β-thalassemia major (TM) and have milder anemia. By definition, they either do not require or only occasionally require transfusions. In some cases, they may remain asymptomatic until adulthood. Clinical features of TI include pallor, mild to moderate jaundice, cholelithiasis, enlargement of the liver and spleen, moderate to severe bone deformities, leg ulcers, and extramedullary masses of hyperplastic erythroid marrow (Cohen et al., 2004; Langer, 1993; Origa, 2017).

Thalassemia carriers are typically clinically asymptomatic, though they may occasionally exhibit mild anemia (Cohen et al., 2004; Langer, 1993; Origa, 2017). In hematologic diagnosis, red blood cell (RBC) indices indicate microcytic anemia. Beta-thalassemia major (TM) is characterized by a hemoglobin (Hb) level of less than 7 g/dL, a mean corpuscular volume (MCV) between 50 and 70 fL, and a mean

corpuscular hemoglobin (MCH) ranging from 12 to 20 pg. Beta-thalassemia intermedia (TI) is identified by Hb levels between 7 and 10 g/dL, MCV greater than 50 but less than 80 fL, and MCH between 16 and 24 pg. Thalassemia minor is marked by decreased MCV and MCH, along with elevated HbA2 levels. Hemoglobin electrophoresis and high-performance liquid chromatography (HPLC) can also detect other hemoglobinopathies that may coexist with β-thalassemia (Origa, 2017).

In affected individuals, peripheral blood smears reveal morphologic changes in RBCs, including microcytosis, hypochromia, anisocytosis, poikilocytosis, and the presence of nucleated RBCs (Figure 2.17). The number of NRBCs correlates with the severity of anemia and is significantly increased following splenectomy. In carriers of thalassemia, there is a reduction in MCV and MCH, along with less pronounced RBC morphologic changes compared to affected individuals. NRBCs are typically not present in carriers (Cohen et al., 2004; Origa, 2017).

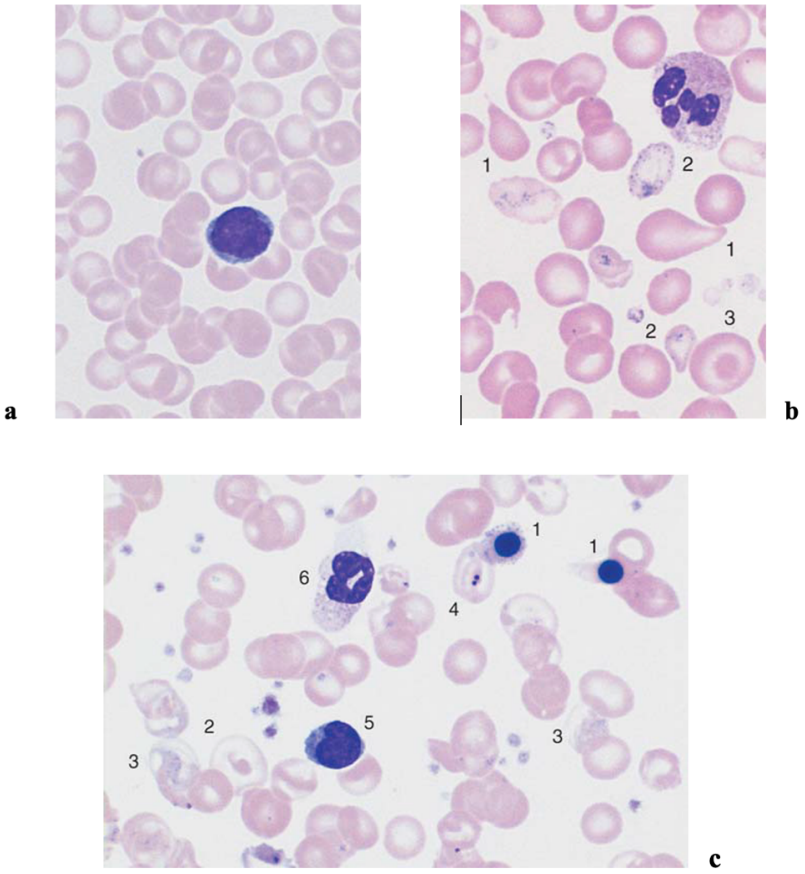

Figure 2.17: Thalassemia. **(a)** Thalassemia minor: often no target cells, but an increase in the number of small erythrocytes. **(b)** More advanced thalassemia minor: strong anisocytosis and poikilocytosis (1), basophilic stippling (2), and sporadic target cells (3). Thalassemia major: erythroblasts (1), target cell (2), polychromatic erythrocytes (3), and Howell–Jolly bodies (4). Lymphocyte (5) and granulocyte (6) (Theml & Diem, 2011).

2.5.1.4 Sickle Cell Anemia

Sickle cell disease (SCD) is a hereditary hemoglobin disorder in which deoxygenated hemoglobin forms long chains within capillaries, causing red blood cells to become sickle-shaped (Figure 2.18). This leads to sickling and hemolysis of red blood cells, resulting in vasoocclusion and ischemia. SCD is marked by recurrent episodes of severe acute pain, acute chest syndrome, and a range of complications including stroke, chronic pain, kidney disease, eye damage (retinopathy), avascular necrosis, priapism, leg ulcers, progressive multi-organ damage, and increased mortality (Brandow & Liem, 2022; Kavanagh et al., 2022).

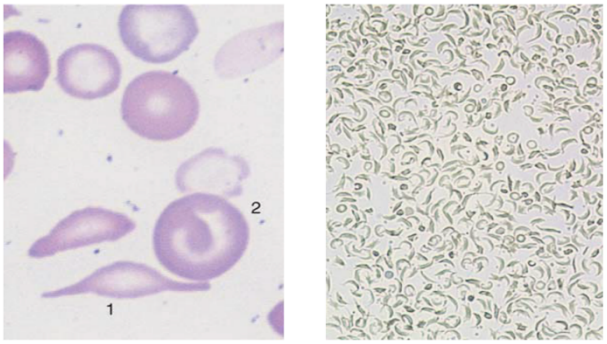

Figure 2.18: Sickle cells. (**a)** Native sickle cells (1) are found only in homozygous sickle cell anemia, otherwise, only target cells (2) are present. (**b)** Sickle cell test under reduced oxygen tension: almost all erythrocytes appear as sickle cells in the homozygous case presented here (Theml & Diem, 2011).

2.5.2 Normocytic Normochromic Anemia

Normocytic normochromic anemia is a type of anemia where red blood cells (RBCs) appear normal in size (normocytic) and hemoglobin content (normochromic) under a microscope. Most cases of normocytic normochromic anemia are secondary to other medical conditions. Common causes include anemia of chronic disease (due to inflammation or cancer), renal failure, endocrine disorders (such as hypothyroidism or hypopituitarism), bone marrow failure (such as pure red-cell aplasia, aplastic anemia, or marrow infiltration), acute blood loss, and polymyalgia rheumatica. This type of anemia usually results from impaired RBC production (Yilmaz & Shaikh, 2024).

Hemolytic anemia is characterized by the premature destruction of red blood cells (RBCs) before their normal 120-day lifespan. It is classified as a normocytic anemia, with a mean corpuscular volume (MCV) ranging from 80 to 100 fL. Hemolytic anemia results in decreased hemoglobin levels, increased hemoglobin breakdown, and

increased effort by the bone marrow to regenerate RBCs. Hemolytic anemia has various causes and can be classified based on acute versus chronic conditions, immune-mediated versus non-immune-mediated factors, intravascular versus extravascular destruction, and inherited versus acquired forms (Baldwin et al., 2024; Phillips & Henderson, 2018).

Intracorpuscular causes involve abnormalities within the RBC itself. These include membranopathies like hereditary spherocytosis (HS) and hereditary elliptocytosis (HE), as well as enzymopathies like glucose-6-phosphate dehydrogenase (G6PD) deficiency and pyruvate kinase deficiency (PKD), which lead to nonspherocytic hemolytic anemias (Baldwin et al., 2024; Phillips & Henderson, 2018).

Extracorpuscular causes involve external factors affecting RBCs, such as mechanical damage, immune-mediated destruction, or infections. RBC transfusions can trigger both acute and delayed hemolytic reactions. Mechanical trauma to RBCs may occur due to microthrombi, fibrin, or valve shearing forces. Infectious agents like malaria and babesiosis also destroy RBCs, and medications such as dapsone, which treat these infections, can cause oxidative damage to RBCs (Baldwin et al., 2024; Phillips & Henderson, 2018).

When hemolysis is suspected, a thorough history is essential, including known medical conditions, medications, personal or family history of hemolytic anemia, and a comprehensive review of systems. The physical examination should focus on identifying associated conditions, such as infections or malignancies (Phillips & Henderson, 2018).

A key laboratory value in diagnosing hemolytic anemia is an elevated reticulocyte count, indicating the bone marrow's increased effort to produce more red blood cells (RBCs). However, because this increase can also occur in conditions like blood loss anemia, it is essential to take a detailed history and evaluate additional lab values, such as elevated lactate dehydrogenase (LDH), decreased haptoglobin, and increased indirect bilirubin, which are typically altered in hemolytic anemia (Baldwin et al., 2024). The standard workup to diagnose normocytic normochromic anemia includes the following steps:

- Complete Blood Count (CBC) with differential: This may need to be repeated if there is an acute drop in blood cell counts. It's important to rule out any dilutional effects, which can occur when a patient receives a large volume of fluids quickly, causing equal reductions across all cell lines (Yilmaz & Shaikh, 2024).
- Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC): These values help assess the size and hemoglobin concentration of red blood cells (Yilmaz & Shaikh, 2024).
- Reticulocyte Count: This is critical for determining the underlying mechanism of anemia. An increased reticulocyte count indicates hemolysis, and other signs of red blood cell destruction, such as elevated lactate dehydrogenase (LDH), increased indirect bilirubin, and low haptoglobin levels, should be considered. A low reticulocyte count points to a hypoproliferative state, such as aplastic anemia, kidney disease, or hypothyroidism (Yilmaz & Shaikh, 2024).
- Peripheral Blood Smear: Examining red blood cell morphology can provide further insights. The absence of schistocytes excludes microangiopathic hemolysis, while the presence of microspherocytes suggests hemolysis (Figure 2.19). Rouleaux formation is often associated with multiple myeloma (Yilmaz & Shaikh, 2024).

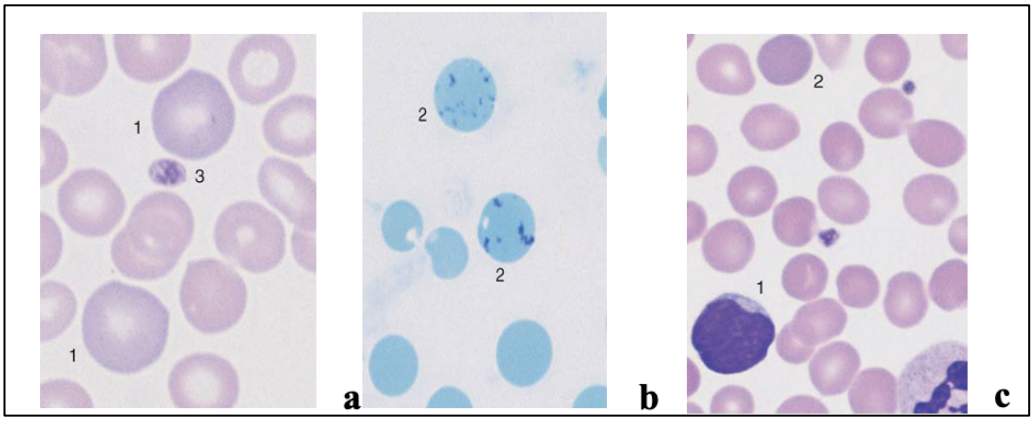

Figure 2.19: Hemolytic anemia. **(a)** Newly formed erythrocytes appear as large, polychromatic erythrocytes (1), Thrombocyte (3), **(b)** after supravital staining reveals spot-like precipitates (reticulocyte = 2). **(c)** lymphocyte (1), microspherocytes (2) (Theml & Diem, 2011).

2.5.3 Macrocytic Anemia

Macrocytic anemia is classified into two main types: megaloblastic and non-megaloblastic. Megaloblastic anemia arises from a deficiency or impaired utilization

of vitamin B12 or folate, while non-megaloblastic anemia can result from conditions such as liver dysfunction, alcoholism, myelodysplastic syndrome (MDS), or hypothyroidism. The common causes of macrocytosis can vary depending on the region and clinical setting (Nagao & Hirokawa, 2017).

Vitamin B12 is primarily found in animal-based foods, with a daily intake ranging from 3-30 μg. The daily requirement is around 1-3 μg, making vitamin B12 deficiency relatively rare unless there is an intestinal or gastric obstruction or a person follows a strict vegetarian diet. Vitamin B12 deficiency is the most common cause of megaloblastic anemia. It can result from inadequate dietary intake, particularly in vegetarians or those with malnutrition, or from malabsorption due to a lack of intrinsic factor (as seen in pernicious anemia or following gastric surgery). Other causes include congenital disorders like transcobalamin II deficiency or exposure to nitrous oxide (Nagao & Hirokawa, 2017).

Symptoms of megaloblastic anemia due to vitamin B12 deficiency include fatigue, headaches, palpitations, and shortness of breath. Neurological symptoms such as dysesthesia and hypoesthesia may also occur. In more severe cases, patients may experience ataxia, decreased proprioception, and diminished vibratory sensation, collectively known as subacute combined degeneration. Notably, neurological symptoms are absent in folate deficiency. Vitamin B12 deficiency may not always result in anemia or macrocytosis, and other signs like Hunter's glossitis and premature graying of hair may also be present (Nagao & Hirokawa, 2017).

Folic acid is found in green leafy vegetables and animal products such as liver. The recommended daily intake for adults is 240 μg, with a higher requirement of around 400 μg per day for pregnant or lactating women, as folate deficiency can increase the risk of congenital neural tube defects during pregnancy. Folic acid is absorbed in the upper jejunum through both passive diffusion and active uptake. Folate deficiency can result from various causes, including poor diet (e.g., malnutrition, alcoholism), malabsorption (e.g., celiac disease, inflammatory bowel disease), increased demands (e.g., pregnancy, lactation, chronic hemolysis), or certain medications (e.g., methotrexate, trimethoprim, phenytoin). Since serum folate levels can vary based on recent dietary intake, measuring RBC folate levels, which indicate tissue folate stores, is considered more reliable. Treatment for folate deficiency typically involves oral

folic acid supplementation, particularly when the deficiency is due to poor diet or increased nutritional needs (Nagao & Hirokawa, 2017).

Pernicious anemia is responsible for 20-50% of vitamin B12 deficiency cases in adults and is linked to autoimmune gastritis, which leads to the destruction of gastric parietal cells and a deficiency in intrinsic factor. This condition is caused by autoimmune metaplastic atrophic gastritis (AMAG), primarily affecting the stomach's body and fundus. In pernicious anemia, autoantibodies targeting gastric parietal cells are present, specifically directed against the hydrogen potassium adenosine triphosphatase (H+/K+-ATPase) proton pump. *Helicobacter pylori* is generally not associated with AMAG (Nagao & Hirokawa, 2017).

A peripheral blood smear in cases of megaloblastic anemia typically shows large red blood cells (megaloblasts) and hypersegmented neutrophils (Figure 2.20). Additionally, poikilocytosis and anisocytosis are frequently observed due to ineffective erythropoiesis. In the bone marrow, megaloblastic changes are evident in erythroblasts and giant metamyelocytes, resulting from impaired nuclear differentiation. The bone marrow evaluation reveals hypercellularity with abnormal maturation and proliferation of red cell precursors, characterized by erythroblasts that exhibit a failure of nuclear maturation (Hariz & Bhattacharya, 2024; Kaferle & Strzoda, 2009).

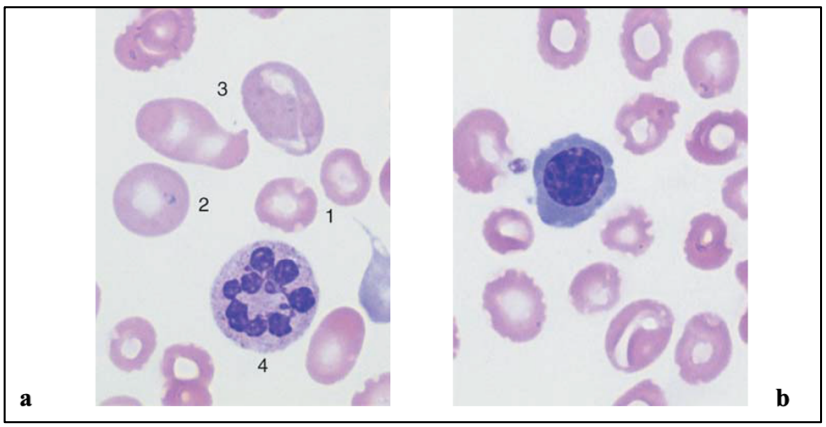

Figure 2.20: Macrocytic anemia. **(a)** Marked anisocytosis. In addition to normal-sized erythrocytes (1), macrocytes (2) and large ovoid megalocytes are seen (3). Hypersegmented granulocyte (4). **(b)** In hyperchromic anemia, red cell precursors may be released into the peripheral blood: here, a polychromatic erythroblast (Theml & Diem, 2011).

2.5.4 Leukocytosis

Leukocytosis, typically defined as a white blood cell (WBC) count exceeding 11,000 per mm^3 (11.0×10^9 per L) in nonpregnant adults. A follow-up CBC with a peripheral smear can provide useful details, such as the types, maturity, and uniformity of WBCs. An elevated white blood cell count has many potential causes, including both malignant and nonmalignant conditions(Riley & Rupert, 2015).

Table 2.6 demonstrates the normal percentage of different types of white blood cells; any alterations in the normal distribution of white blood cell (WBC) types can point to specific causes of leukocytosis.

Table 2.6: Normal White Blood Cells distribution. (Source: (Riley & Rupert, 2015))

White blood cell line	Normal percentage of total leukocyte count (%)
Neutrophils	40 – 60
Lymphocytes	20 – 40
Monocytes	2 – 8
Eosinophils	1 – 4
Basophils	0.5 – 1

2.5.4.1 Non-Malignant Leukocytosis

The most common form of leukocytosis is neutrophilia (Figure 2.21), which is characterized by an increase in the absolute number of mature neutrophils to more than 7,000 per mm^3 (7.0×10^9 per L). This can result from infections, stress, chronic inflammation, medication use, and various other factors (Riley & Rupert, 2015; Tahir & Zahra, 2024).

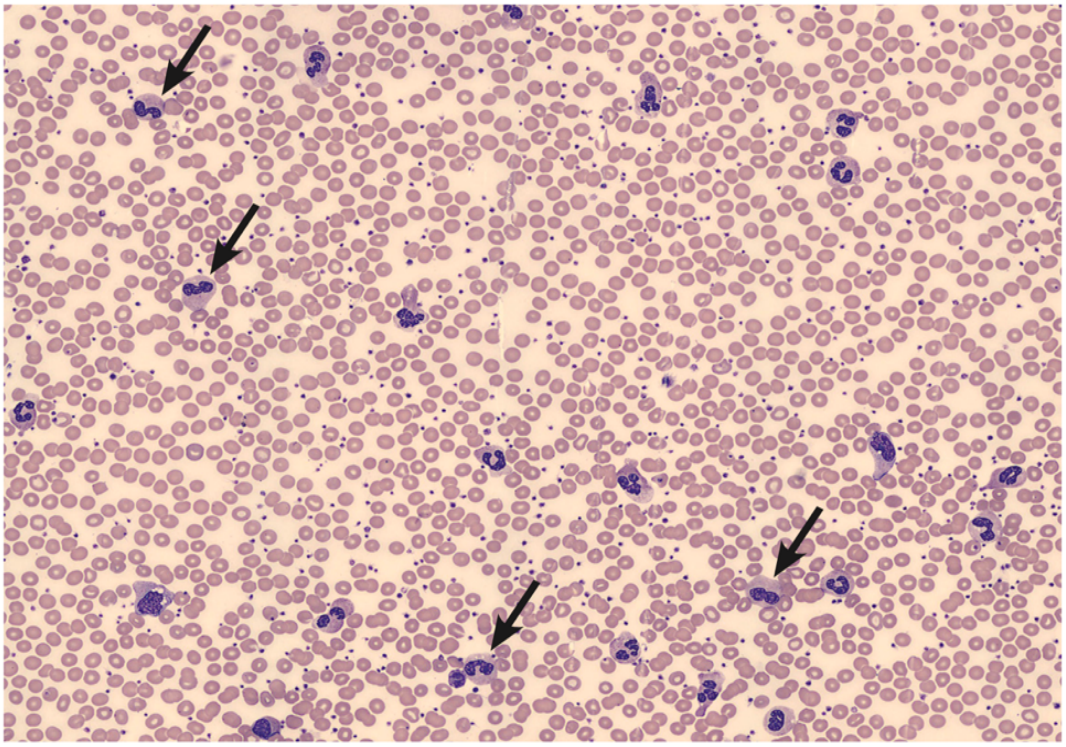

Figure 2.21: Neutrophilia post-splenectomy with sepsis (Riley & Rupert, 2015).

Lymphocytosis (Figure 2.22), where lymphocytes comprise over 40% of the WBC count or the absolute count exceeds 4,500 per mm^3 (4.5×10^9 per L). Reactive lymphocytes (Figure 2.23) can be seen in conditions such as pertussis (whooping cough), syphilis, viral infections, chickenpox, hypersensitivity reactions, and certain leukemias or lymphomas. Lymphocytosis is more likely to be benign in children compared to adults. Monocytosis, defined by monocytes accounting for more than 8% of the WBC count or an absolute count over 880 per mm^3 (0.88×10^9 per L), can be triggered by Epstein-Barr virus, tuberculosis, fungal infections, autoimmune diseases, splenectomy, protozoan or rickettsia infections, and malignancies (Riley & Rupert, 2015).

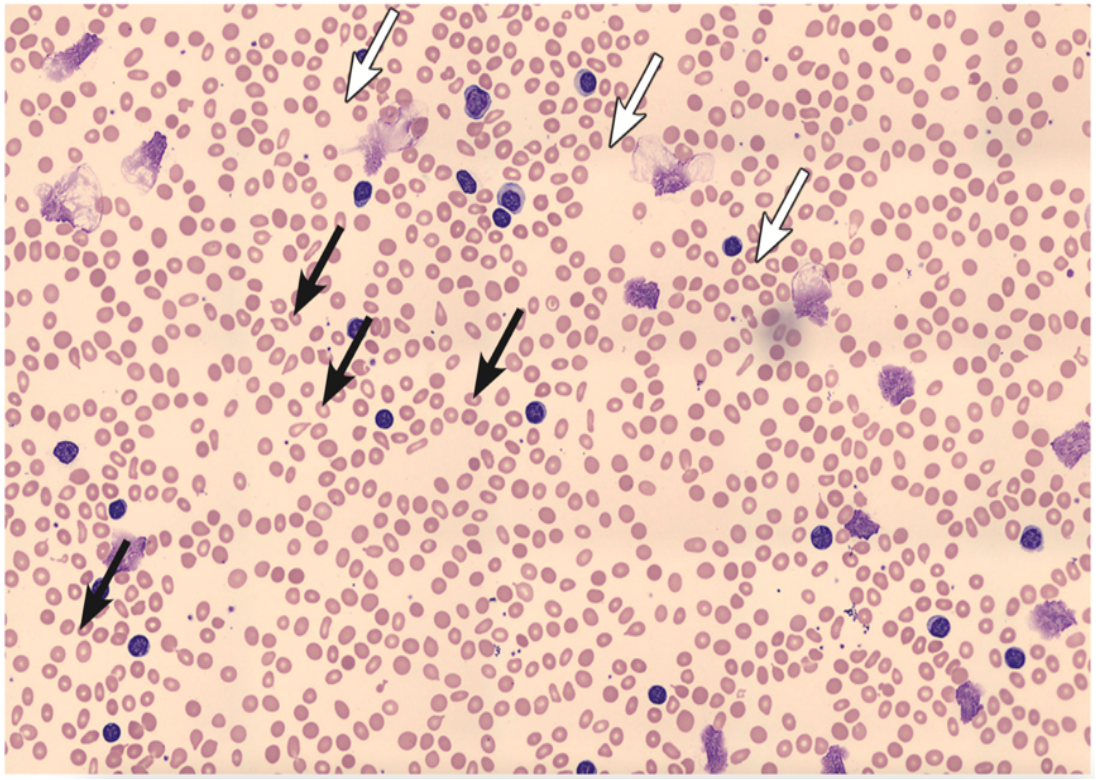

Figure 2.22: Lymphocytosis, many mature lymphocytes (small nuclei near the size of a red blood cell with thin rim cytoplasm; black arrows) and many smudge cells (white arrows) (Riley & Rupert, 2015).

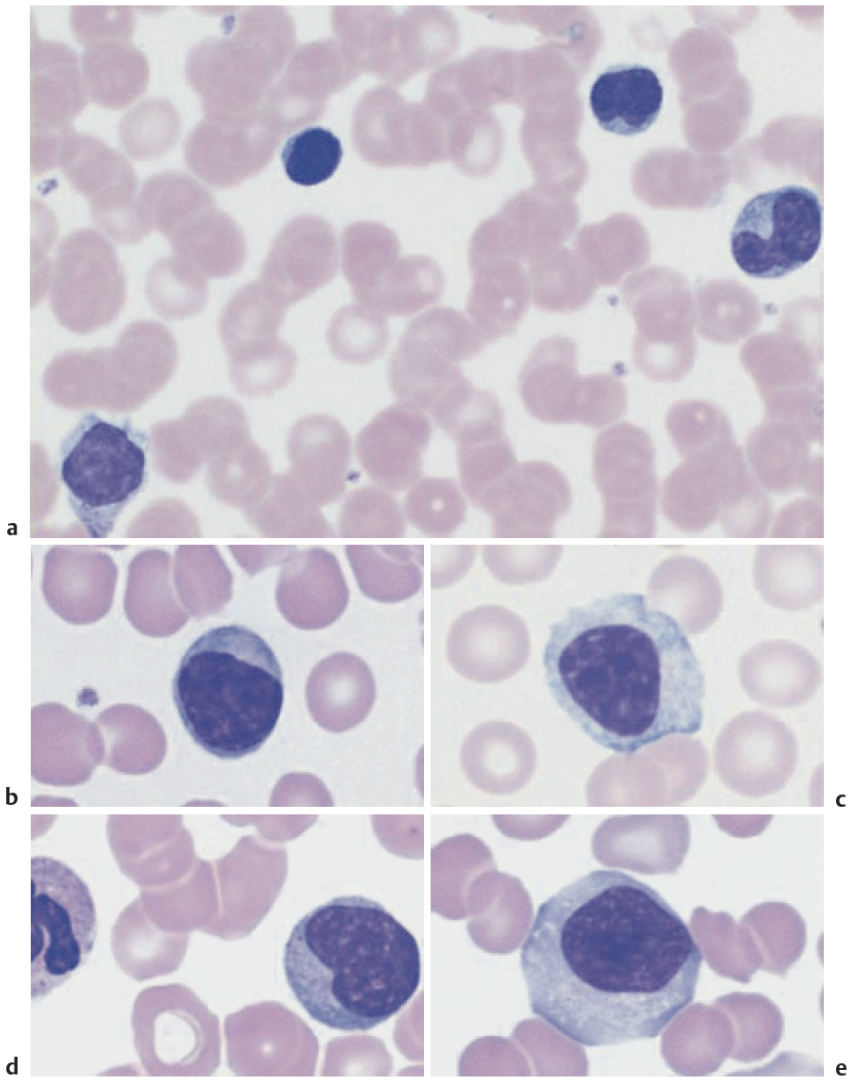

Figure 2.23: Reactive Lymphocytes. **(a-e)** Wide variability of the lymphocytes in a lymphotropic infection. Some of the cells may resemble myelocytes, but their chromatin is always denser than myelocyte chromatin (Theml & Diem, 2011).

Eosinophilia (Figure 2.24), defined as an absolute eosinophil count exceeding 500 per mm^3 (0.5×10^9 per L), is relatively rare but may indicate allergic conditions such as asthma, urticaria, atopic dermatitis, or eosinophilic esophagitis. It can also result from drug reactions, skin disorders, malignancies, connective tissue diseases, idiopathic hypereosinophilic syndrome, or parasitic infections, particularly those caused by tissue parasites (e.g., helminths) rather than gut-lumen parasites. Basophilia, with a basophil count above 100 per mm^3 (0.1×10^9 per L), is uncommon and rarely leads to leukocytosis on its own. However, it may occur in allergic or inflammatory conditions and in chronic myelogenous leukemia (Kovalszki & Weller, 2016; Riley & Rupert, 2015).

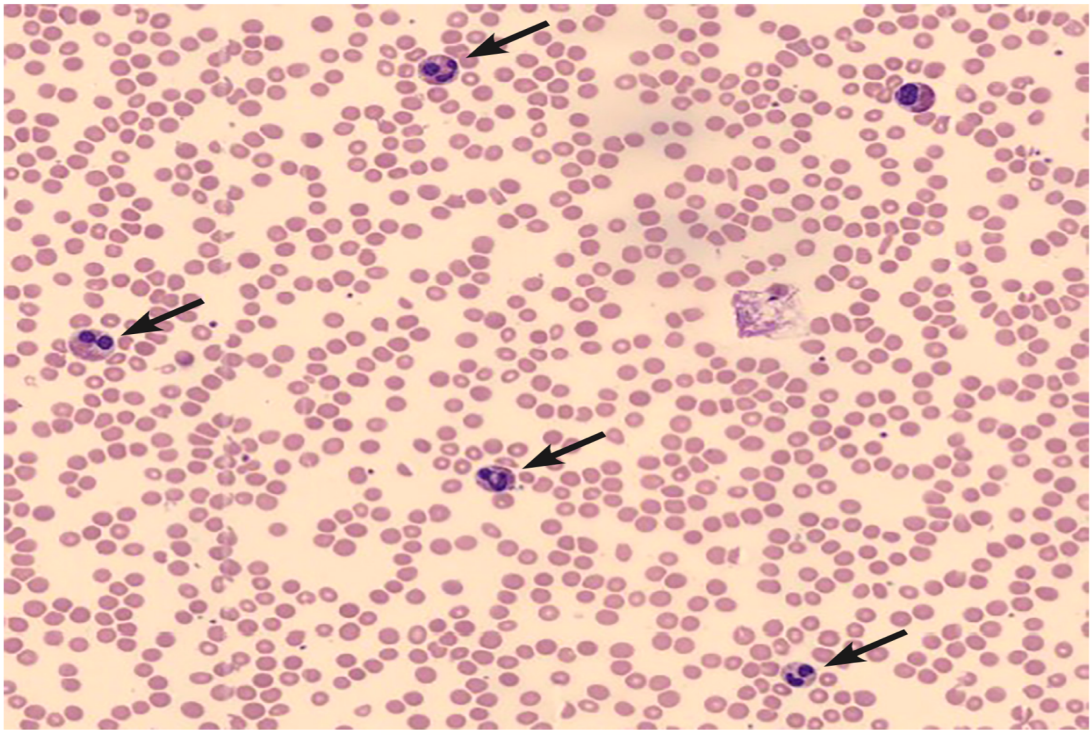

Figure 2.24: Eosinophilia in malignancy (Riley & Rupert, 2015).

2.5.4.2 Malignant Leukocytosis

Leukocytosis can be an early indicator of a malignant condition, such as acute or chronic leukemia, or a myeloproliferative disorder like polycythemia vera, myelofibrosis, or essential thrombocytosis. Additionally, many solid tumors may cause leukocytosis at levels resembling a leukemoid reaction, either by direct involvement of the bone marrow or through the production of granulocyte colony-stimulating factors (G-CSF) or granulocyte macrophage colony-stimulating factors (GM-CSF) (Riley & Rupert, 2015).

A complete blood count typically shows leukocytosis along with other abnormal elevations or decreases in cell lines. Patients suspected of having leukemia should be promptly referred to a hematologist-oncologist. The diagnosis is confirmed through further analysis of the bone marrow or peripheral blood (Davis et al., 2014).

2.5.5 Leukemia

Leukemia is characterized by the clonal proliferation of hematopoietic stem cells in the bone marrow. The four main subtypes that primary care physicians are most likely

to encounter are acute lymphoblastic leukemia, acute myelogenous leukemia, chronic lymphocytic leukemia, and chronic myelogenous leukemia (Davis et al., 2014).

2.5.5.1 Acute Leukemia

Leukemia in children predominantly manifests as Acute Lymphoblastic Leukemia (ALL), which accounts for approximately 75% to 100% of cases. Common presenting signs and symptoms include fever, lethargy, and bleeding. About one-third of children may experience musculoskeletal symptoms, particularly in the spine and long bones, as well as enlarged liver or spleen and lymphadenopathy. Central nervous system involvement is present in about 7% of children at the time of diagnosis (Davis et al., 2014).

In adults, Acute Myelogenous Leukemia (AML) accounts for 80% of acute leukemia cases. Adults typically present with constitutional symptoms such as fever, fatigue, and weight loss. They may also exhibit symptoms related to anemia, including shortness of breath or chest pain, as well as symptoms associated with thrombocytopenia, such as excessive bruising, nosebleeds, or heavy menstrual periods in women. Adults are less likely to report bone pain. While hepatosplenomegaly and lymphadenopathy are rare in adults with AML, they are present in about 50% of adults with ALL. Central nervous system involvement occurs in approximately 5% to 8% of adults with acute lymphoblastic leukemia (Davis et al., 2014).

Acute leukemia should be suspected when a peripheral blood smear or bone marrow specimen shows an abundance of blast cells (Figure 2.25), which are the earliest forms of hematopoietic precursor cells. Acute myelogenous leukemia (AML) is typically characterized by the presence of Auer rods in a peripheral smear. However, since Auer rods are not frequently observed, immunophenotyping via flow cytometry and cytogenetic testing are necessary to differentiate between subtypes of acute leukemia, such as AML and acute lymphoblastic leukemia (ALL) (Davis et al., 2014; Pelcovits & Niroula, 2020).

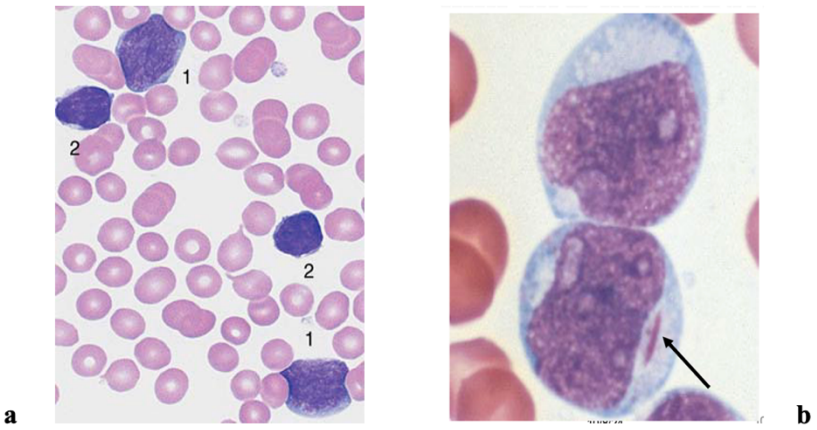

Figure 2.25: Blast cells in Acute leukemia**.** **(a)** Undifferentiated blast with dense, fine chromatin nucleolus, and narrow basophilic cytoplasm without granules (1), lymphocytes (2). **(b)** Blast cell with Auer rod found in AML (Theml & Diem, 2011).

2.5.5.2 Chronic Leukemia

Chronic leukemia subtypes primarily occur in adults, and patients may be asymptomatic at the time of diagnosis. Approximately 50% of individuals with Chronic Lymphocytic Leukemia (CLL) and 20% of those with Chronic Myelogenous Leukemia (CML) receive their diagnosis incidentally when marked leukocytosis is identified on a complete blood count conducted for an unrelated issue. Constitutional symptoms are less frequent, occurring in about 15% of patients with CLL and in approximately one-third of those with CML. Common physical examination findings in individuals with CLL include hepatosplenomegaly and lymphadenopathy. In patients with CML, splenomegaly is also common. Bleeding and bruising are less common presenting features in chronic leukemia subtypes (Davis et al., 2014).

The diagnosis of chronic lymphocytic leukemia (CLL) is established through the clonal expansion of at least 5,000 B lymphocytes per µL (5.0×10^9 per L) in the peripheral blood, which is confirmed by immunophenotyping. Although a bone marrow specimen is not necessary for diagnosing CLL, it may be obtained to assess the extent of marrow involvement for prognostic purposes (Davis et al., 2014).

In contrast, the diagnosis of chronic myelogenous leukemia (CML) requires cytogenetic or molecular testing of the bone marrow or peripheral blood to identify a specific abnormality known as the Philadelphia chromosome or the BCR-ABL1 fusion gene. This fusion gene arises from a reciprocal translocation between chromosomes 9 and 22, disrupting normal cell regulatory processes in the bone

marrow. The Philadelphia chromosome is present in 95% of patients with CML, while the remaining 5% exhibit different chromosomal rearrangements but still produce the abnormal BCR-ABL1 fusion gene (Chiorazzi et al., 2005; Davis et al., 2014).

During the course of chronic myelogenous leukemia (CML), with or without treatment, regular monitoring of the differential blood smear is crucial, as the proportion of blasts and promyelocytes tends to increase over time. When the combined fraction of blasts and promyelocytes reaches 30%, along with hemoglobin levels dropping below 10 g/dL and a platelet count falling below 100,000/µL, an impending acute blast crisis should be suspected. This blast crisis (Figure 2.26) is often accompanied or preceded by a significant rise in basophil count. As blast cells continue to increase, usually becoming resistant to treatment, the clinical presentation may resemble that of acute leukemia. If the disease remained in the "latent" chronic phase without medical intervention, signs of CML leading to the blast crisis include spleen enlargement, slight eosinophilia and basophilia, occasional normoblasts, and a predominance of myeloblasts (Theml & Diem, 2011).

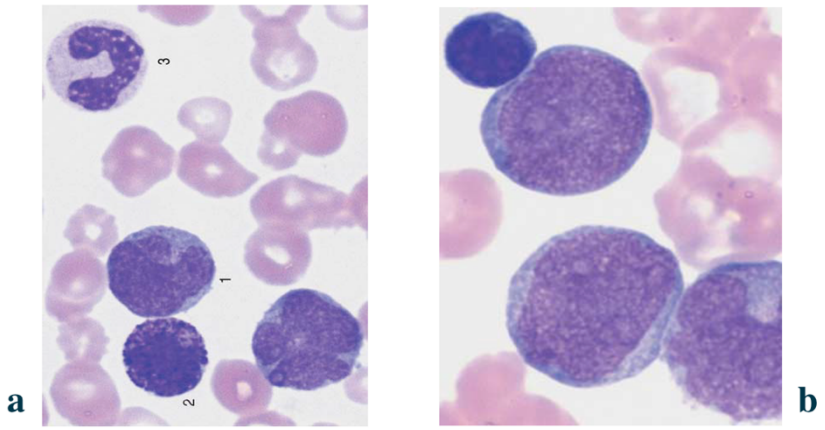

Figure 2.26: Acute blast crisis in CML. **(a)** Myeloblasts (1) with somewhat atypical nuclear lobes. Basophilic granulocyte (2) and band granulocyte (3). Thrombocytopenia. The proliferation of basophilic granulocytes often precedes the blast crisis. **(b)** Myeloblasts in an acute CML blast crisis (Theml & Diem, 2011).

Smear or smudge cells (SCs) (Figure 2.27), are fragments of chronic lymphocytic leukemia (CLL) B-cells seen in blood smears of CLL patients. It has been observed that the formation of smudge cells is related to the amount of the cytoskeletal protein vimentin present in leukemic cells. The percentage of smudge cells in a routine blood smear serves as an independent prognostic factor in CLL, with patients having a

higher percentage of smudge cells generally experiencing longer survival (Sall et al., 2022).

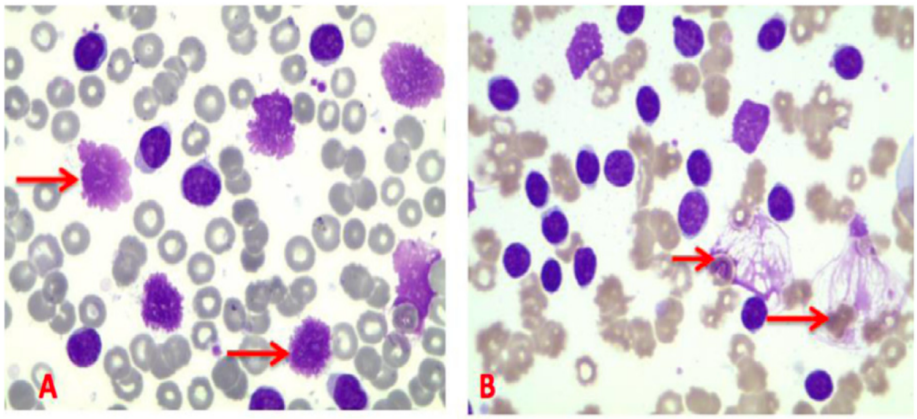

Figure 2.27: Smudge cells**.** Blood smears of different CLL patients. **(A and B)**: different forms of SC (arrows) (Sall et al., 2022).

When anemia is present along with moderately elevated (or sometimes reduced) leukocyte counts, thrombocytopenia or thrombocytosis, clinically apparent splenic tumors, a left shift with occasional myeloblasts, and eosinophilia, osteomyelosclerosis should be considered (Figure 2.28). This suspicion is further supported by the presence of a significant proportion of red cell precursors (normoblasts) in the differential blood analysis. Additionally, BCR-ABL gene analysis will be negative in such cases (Theml & Diem, 2011).

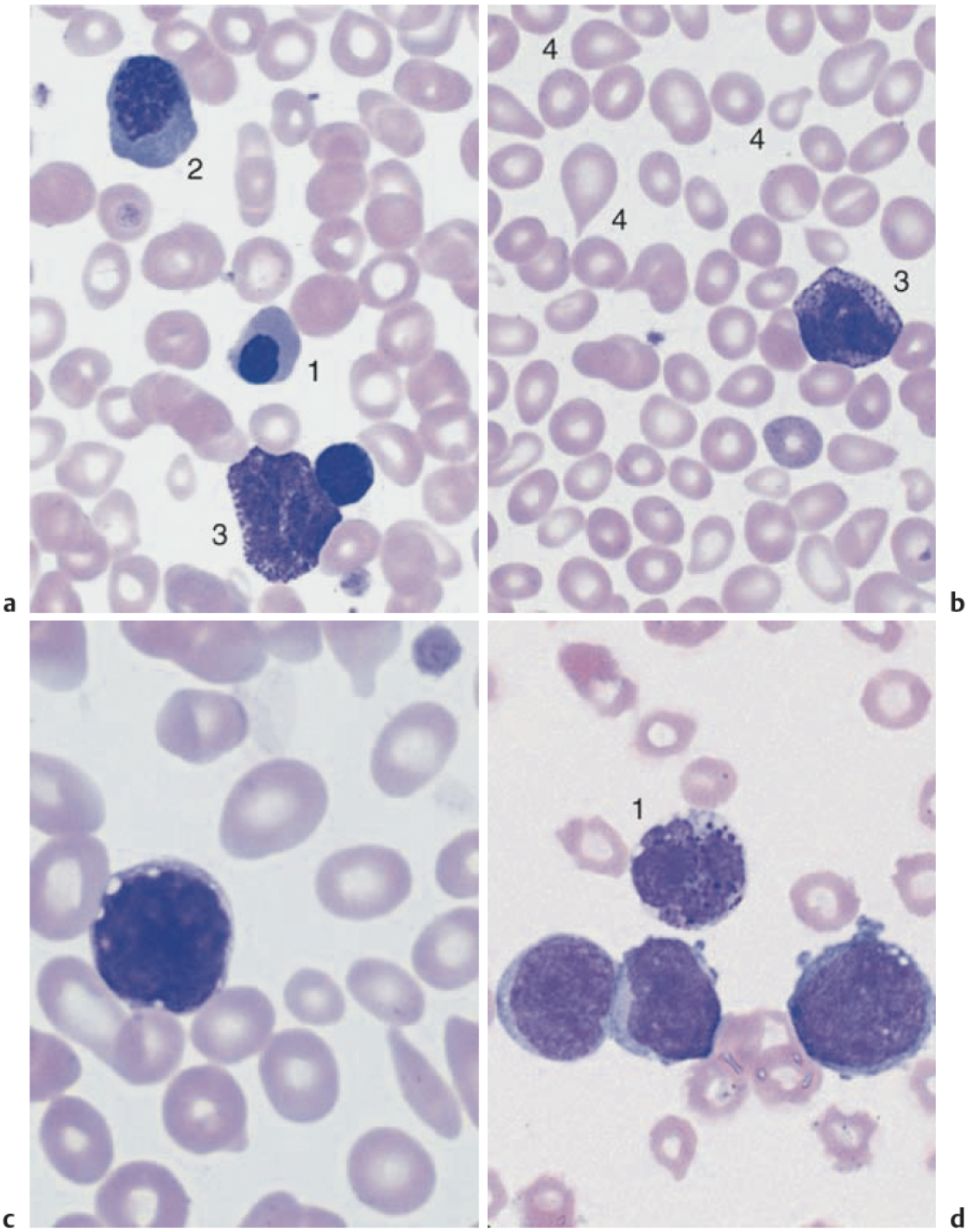

Figure 2.28: Osteomyelosclerosis (OMS). **(a and b)** Screening of blood cells in OMS: red cell precursors (**(1)** orthochromatic erythroblast, and basophilic erythroblast **(2)**), basophilic granulocyte (3), and teardrop cells (4). **(c)** Sometimes small, dense megakaryocyte nuclei are also found in the blood stream in myeloproliferative diseases. **(d)** Blast crisis in OMS: myeloblasts and segmented basophilic granulocytes (1) (Theml & Diem, 2011).

2.5.6 Platelets Abnormalities

2.5.6.1 Thrombocytopenia

Thrombocytopenia, characterized by a platelet count below 150 x 10^9/L (or 100 x 10^9/L in some cases). Thrombocytopenia is classified as mild when platelet counts range between 70 and 150 x 10^3/μL and severe when they fall below 20 x 10^3/μL. Patients with platelet counts above 50 x 10^3/μL are often asymptomatic. Those with counts between 30 and 50 x 10^3/μL rarely exhibit purpura but may experience excessive bleeding with trauma. When counts are between 10 and 30 x 10^3/μL, bleeding can occur with minimal trauma. Platelet counts below 10 x 10^3/μL increase the risk of spontaneous bleeding, petechiae, and bruising. Spontaneous bleeding, such as mucosal, intracranial, gastrointestinal, or genitourinary bleeding, becomes more likely when counts drop below 5 x 10^3/μL and is considered a hematologic emergency (Gauer & Braun, 2012; Smock & Perkins, 2014).

Thrombocytopenia can arise from various underlying mechanisms (Gauer & Braun, 2012; Smock & Perkins, 2014; Theml & Diem, 2011), including:

- Decreased Platelet Production: This can occur due to bone marrow disorders, infections, nutritional deficiencies (like vitamin B12 or folate), or exposure to toxins or chemotherapy.
- Increased Platelet Destruction: Conditions such as immune thrombocytopenia (ITP), hemolytic uremic syndrome (HUS), or disseminated intravascular coagulation (DIC) can accelerate platelet destruction.
- Increased Splenic Sequestration: This occurs in cases of splenomegaly, where platelets are trapped in the enlarged spleen.
- Dilutional: Following massive transfusions, the platelet concentration can become diluted, leading to thrombocytopenia.

For diagnostic evaluation, CBC provides the current platelet count and other important hematologic values. Peripheral Blood Smear helps in identifying morphological abnormalities in the platelets, like clumping or size variations (see Figure 2.29) (Smock & Perkins, 2014).

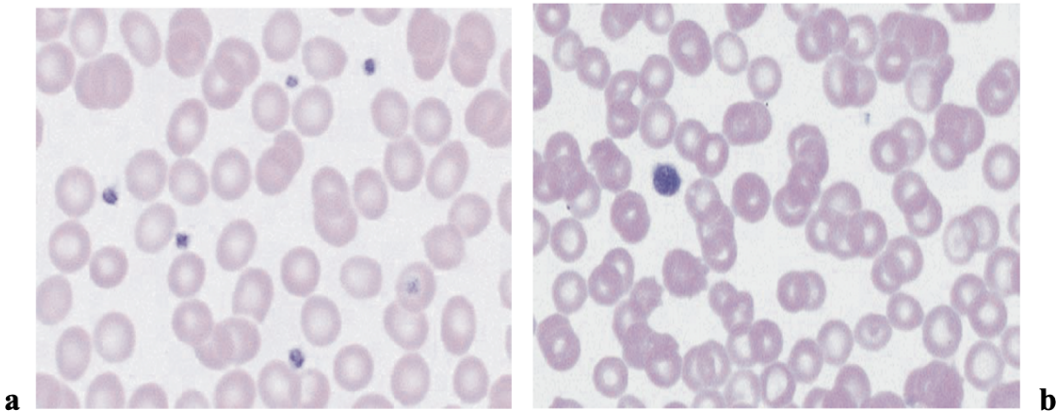

Figure 2.29: Forms of thrombocytopenia. **(a)** This blood smear shows normal size and density of thrombocytes. **(b)** In this blood smear thrombocyte density is lower and size has increased (Theml & Diem, 2011).

Pseudothrombocytopenia (Figure 2.30), where EDTA used in blood collection causes platelet clumping, can give a falsely low platelet count. In such cases, a repeat test using a citrate tube (blue top tube) can help rule out this artifact (Smock & Perkins, 2014).

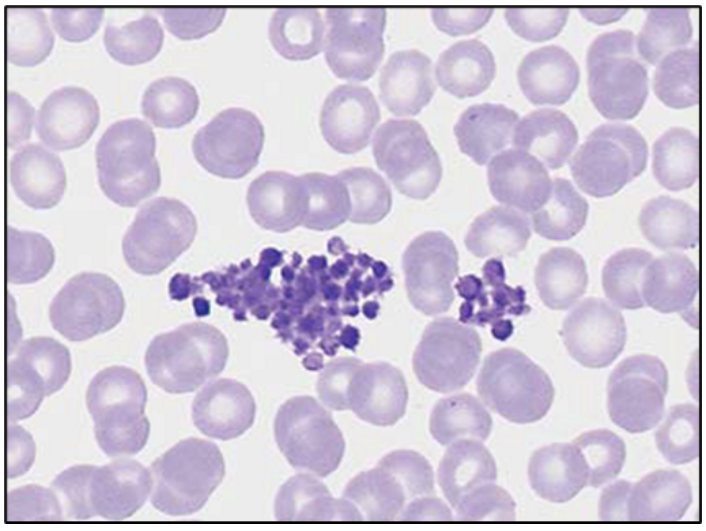

Figure 2.30: Pseudothrombocytopenia. The thrombocytes are not lying free and scattered around, but agglutinated together, leading to a reading of thrombocytopenia from the automated blood analyzer (Theml & Diem, 2011).

A falsely low automated platelet count also can occur when platelets attach to white blood cells and are thus excluded from the reported count. A rare phenomenon where platelets form clusters around the WBC is known as platelet satellitism (Figure 2.31). This phenomenon has been associated with various inflammatory conditions, such as autoimmune diseases, acute trauma, burns, and infections, though it can also occur in healthy individuals (Tyrrell et al., 2022).

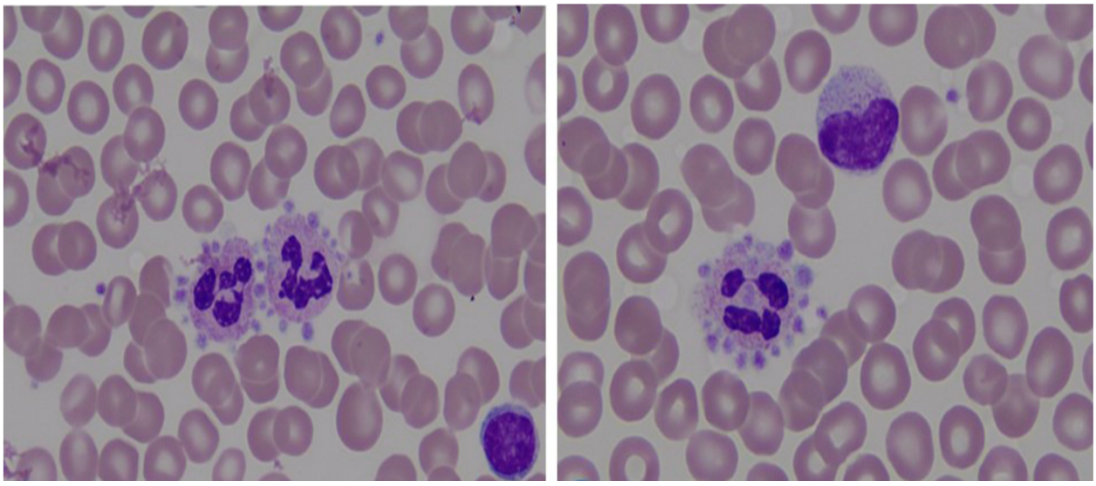

Figure 2.31: Platelets satellitism**.** A rare phenomenon where platelets form clusters around a white blood cell (Tyrrell et al., 2022).

2.5.6.2 Thrombocytosis

Thrombocytosis, also known as thrombocythemia, is generally defined as a platelet count exceeding the normal upper limit, typically accepted as <450 x 10^3/µL. Platelet levels between (450 and 700) x 10^3/µL are classified as mild, (700 to 900) x 10^3/µL as moderate, (900 to 1,000) x 10^3/µL as severe, and counts over 1,000 x 10^3/µL are considered extreme thrombocytosis. The primary complications of thrombocytosis include bleeding and thrombotic events, although it often presents without symptoms. Platelet counts above 1,500 x 10^3/µL significantly raise the risk of bleeding. The most common cause of elevated platelet counts is reactive (secondary) thrombocytosis, which is a normal physiological response to inflammation or surgery. In secondary thrombocytosis, the increase in platelets results from an external factor, such as acute or chronic inflammation, that stimulates platelet production. Conditions like bacterial and viral infections, iron deficiency, hemolytic anemia, tissue damage, asplenia, cancers, autoimmune disorders, and certain medications can trigger secondary thrombocytosis. It is frequently observed in children with various medical conditions (Babacan & Şenol, 2023).

Essential thrombocythemia (ET), by contrast, is a type of myeloproliferative disorder characterized by an increased number of platelets, often accompanied by other features of myeloproliferative diseases, such as leukocytosis and splenomegaly, which can vary in severity. Severe thrombocytosis can also be observed in conditions like osteomyelosclerosis, polycythemia vera, and chronic myeloid leukemia (Brière, 2007; Theml & Diem, 2011). To differentiate essential thrombocythemia from other causes

of high platelet counts, the following diagnostic criteria have been proposed (Theml & Diem, 2011):

1. Platelet count >600 x 10^3/µL.
2. Normal RBC mass or hemoglobin levels: <18.5 g/dL in men and <16.5 g/dL in women.
3. No significant bone marrow fibrosis.
4. Absence of splenomegaly.
5. No leukoerythroblastic changes in the complete blood count.
6. No cytogenetic or morphological features of myelodysplasia.
7. Exclusion of secondary thrombocytosis causes (e.g., iron deficiency, inflammation, malignancy, trauma).

Large platelets are often observed in the peripheral blood smear, although this finding can also be seen in polycythemia vera and osteomyelosclerosis. Bone marrow examination typically reveals a significant increase in megakaryocyte numbers, with the megakaryocytes frequently clustering and showing hypersegmented nuclei (see Figure 2.32) (Theml & Diem, 2011).

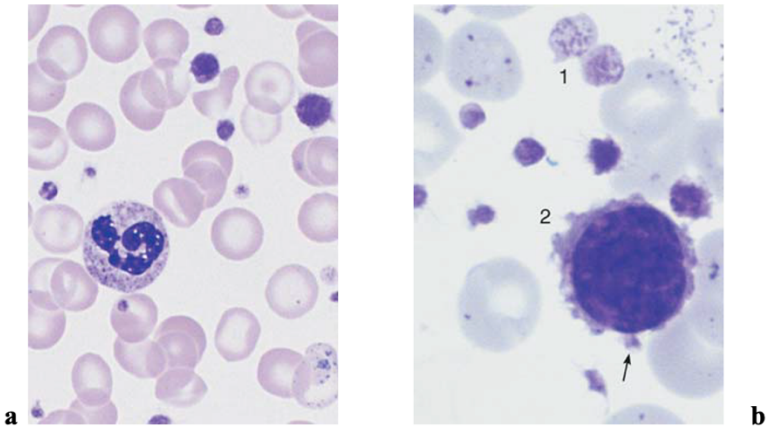

Figure 2.32: Essential thrombocythemia. **(a)** Increased thrombocyte density and marked anisocytosis in essential thrombocythemia. **(b)** Large thrombocytes (1) and a micro(mega)karyocyte nucleus (2) in essential thrombocythemia (Theml & Diem, 2011).

Pseudo-thrombocytosis, or a falsely elevated platelet count, is less common than pseudothrombocytopenia. In rare cases, red blood cell fragments, microspherocytes, protein clumps, cytoplasmic remnants of white blood cells, or lipid droplets may be miscounted as platelets by certain automated analyzers. Microspherocytosis and red

blood cell fragmentation, leading to pseudo-thrombocytosis, have been notably reported in patients with severe burns (Tyrrell et al., 2022).

2.5.7 COVID-19 and Peripheral Blood Cell Morphology

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has resulted in significant global healthcare challenges. While it was initially identified as a respiratory infection, recent research has shown that COVID-19 can present a wide variety of clinical symptoms, ranging from mild to moderate upper respiratory infections to severe systemic conditions affecting the immune, gastrointestinal, cardiovascular, neurological, immunological, and hematopoietic systems. The hematological impacts of COVID-19 continue to receive attention, particularly in public health (Karapetyan et al., 2024; Sarna et al., 2022).

COVID-19 patients may experience various blood clotting disorders. The infection also triggers inflammatory responses that release immature blood cells from the bone marrow. Atypical lymphocytes and immature cells have been detected in both peripheral blood and bronchoalveolar lavage (BAL) samples. As well, significant changes in RBC morphology have been noted (see Figure 2.33), such as sickle cells, spherocytes, echinocytes, stomatocytes, and elliptocytes. These abnormal erythrocytes may suggest autoimmune hemolytic anemia and an immune response to the virus (Karapetyan et al., 2024).

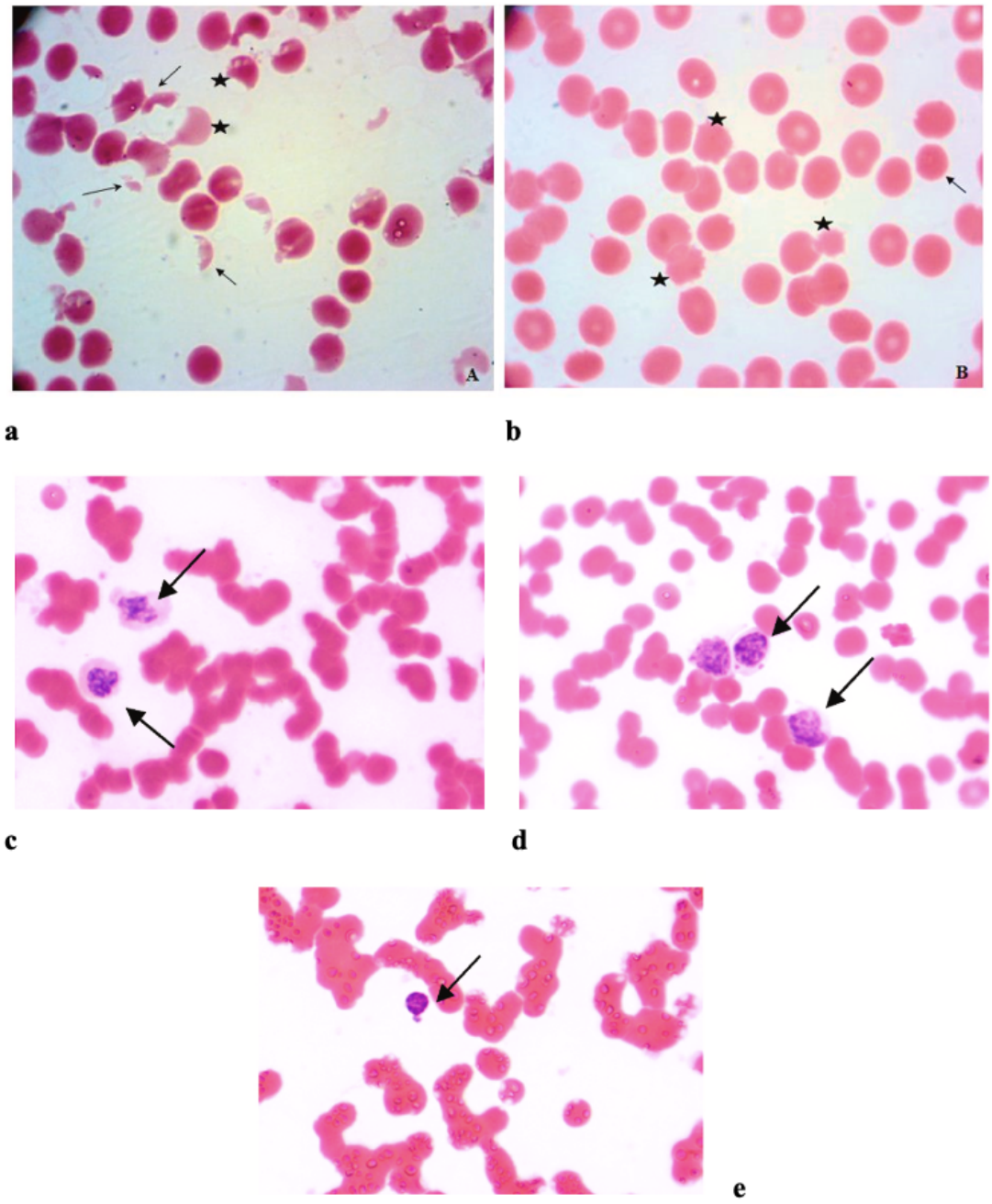

Figure 2.33: Erythrocytes in COVID-19**.** **(a)** sickles (asterisk), schistocytes (fragments) (arrows), meniscocytes (crescent-shaped erythrocytes) (arrowhead), **(b)** echinocytes (asterisk), spherocytes (arrowhead). **(c)** karyorrhexis (fragmentation of nucleus and break up of chromatin), **(d)** micronuclei within the lymphocytes, **(e)** protrusion (magnification 400x) (Karapetyan et al., 2024).

COVID-19 infection is often associated with alterations in platelet count. Thrombocytopenia has been reported in up to 40% of cases and is an important indicator of both morbidity and mortality. The reduction in platelets count may be caused by the infection itself (septicemia), disseminated intravascular coagulation (DIC), medications, or COVID-19-associated immune thrombocytopenic purpura

(ITP). However, a rare and frequently overlooked cause of thrombocytopenia is pseudothrombocytopenia (see Figure 2.34) (Van Dijck et al., 2021).

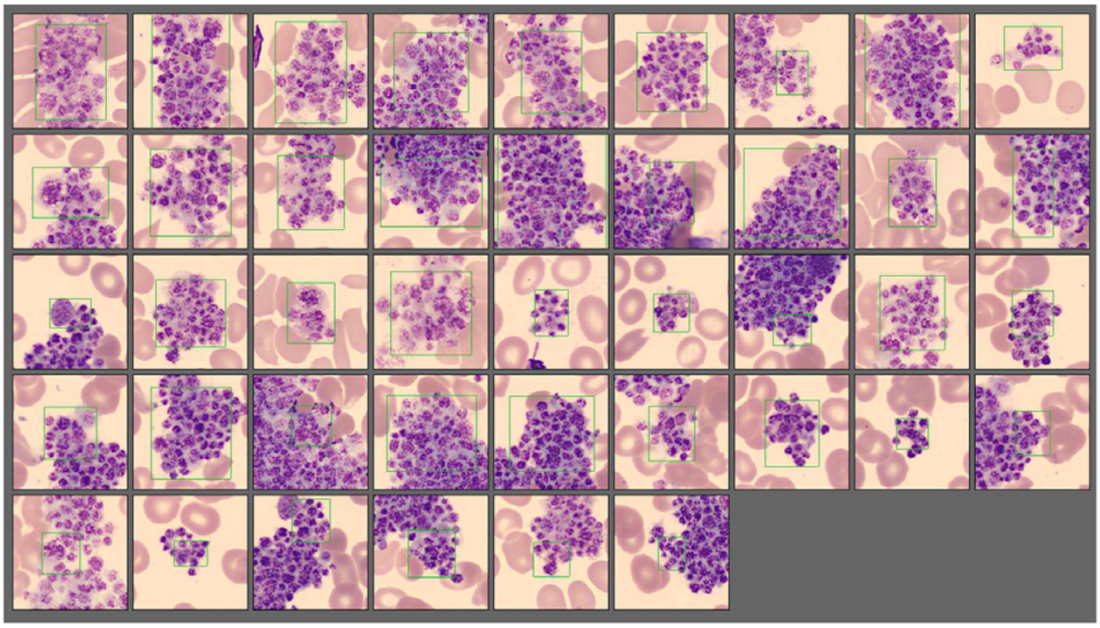

Figure 2.34: Platelets agglutination in COVID-19**.** Peripheral blood smear showing platelet agglutination in EDTA in our COVID-19-infected patient with pseudothrombocytopenia (Van Dijck et al., 2021).

2.5.8 Other Uses of Blood Smears

2.5.8.1 Reticulocyte count

The visual counting of reticulocytes is a commonly employed and acknowledged examination in our labs (Ali et al., 2010). It furnishes essential insights into the categorization and development of anemia. The reticulocyte count serves as an indicator of the erythropoietic activity taking place in the bone marrow. These immature red blood cells, known as reticulocytes, are generated in the bone marrow and subsequently enter the bloodstream, where they undergo maturation into fully developed red blood cells within a span of 1 to 2 days (Ali et al., 2010; Rai et al., 2024).

Reticulocyte counts were obtained by creating smears through the mixing of an equal amount of EDTA-blood and supravital stain (new methylene blue or brilliant crysel blue) in a polystyrene tube, followed by incubation for 20 minutes at 37 °C. Subsequently, blood films were generated on glass slides, allowed to dry, and reticulocytes were counted using a microscope with a × 100 objective lens. The

analysis involved examining a minimum of 1000 red blood cells, and the count was expressed as a percentage (Ali et al., 2010; Koepke & Koepke, 1986; Simionatto et al., 2010).

2.5.8.2 Blood Parasites

Microscopy is still fundamental in laboratory practice for the detection of infections caused by blood and tissue parasites. The analysis of peripheral blood smears, both thick and thin, stained with Giemsa or suitable alternatives, is employed to identify and detect species such as Plasmodium, Babesia, Trypanosoma, Brugia, Mansonella, and Wuchereria (Rosenblatt et al., 2009). Figure 2.35 exhibits an example of a blood parasite (Plasmodium falciparum), which was diagnosed by peripheral blood smear.

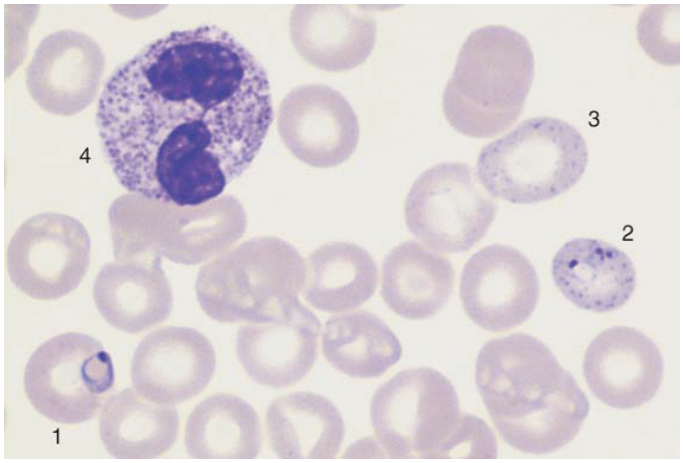

Figure 2.35: Plasmodium falciparum trophozoite**.** Simple signet-ring form (1), double-invaded erythrocyte with basophilic stippling (2). Erythrocyte with basophilic stippling without plasmodium (3) and segmented neutrophilic granulocyte with toxic granulation (4) (Theml & Diem, 2011).

2.6 Proficiency Testing

The beginnings of proficiency testing (PT) in clinical laboratories may be traced back to the late 1940s, when American physician William Sunderman introduced the concept (Sunderman Sr, 1992). There has been increasing interest in PT. Figure 2.36 shows the number of scientific publications each year related to PT in medical laboratories in the period between 1970 and 2000 (Rej, 2002).

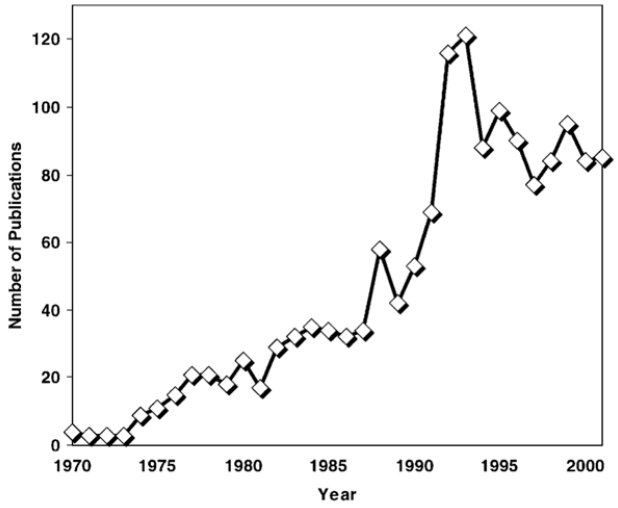

Figure 2.36: Number of scientific papers related to PT (Rej, 2002).

Proficiency testing (PT) stands as a fundamental element within laboratory quality management systems, assessing a laboratory's competence in utilizing specific instruments or accurately applying certain methods. This process offers a comprehensive understanding of measurement procedures, enabling each laboratory to evaluate its test outcomes in comparison to a relevant peer group (consisting of other laboratories employing identical or similar instruments and methods) (Halim, 2013).

The terms proficiency testing (PT) and external quality assessment (EQA) encompass multiple formal definitions. Generally, the PT is more commonly employed in North America, often carrying regulatory or legal attributes. The EQA constitutes a broader practice, wherein proficiency testing serves as one component and is typically seen as an educational tool and a means of self-assessment. Both terms are colloquially used interchangeably, as evidenced by a cursory examination of biomedical and analytical literature and authoritative documents. Assessment of laboratory accuracy and imprecision is a basic function of all external quality assurance schemes (Rej, 2002).

2.6.1 Clinical Laboratory Improvement Amendments

In 1988, the United States Congress established the Clinical Laboratory Improvement Amendments (CLIA) to ensure quality standards applicable to all laboratory-based testing. The primary objective was to guarantee the accuracy, reliability, and timeliness of patient test results, irrespective of the testing location (Rivers et al.,

2005). The CLIA of 1988 (CLIA'88) serve as the basis for accredited external quality control programs (Rej, 2002; Rivers et al., 2005).

Starting in 1994, clinical laboratories performing were required by the CLIA'88 to register and participate in a PT program approved by the Centers for Medicare & Medicaid Services (CMS) (Halim, 2013; Rivers et al., 2005). The CMS is part of three agencies within the Department of Health and Human Services (DHHS), which are the CMS, Disease Control and Prevention (CDC), and Food and Drug Administration (FDA). The CMS has a primary responsibility for managing the CLIA program (Rivers et al., 2005). A laboratory can choose to be accredited by one of six CMS-approved accrediting organizations, including: American Association of Blood Banks (AABB), American Osteopathic Association (AOA), American Society of Histocompatibility and Immunogenetics (ASHI), College of American Pathologists (CAP), Commission on Office Laboratory Accreditation (COLA), Joint Commission on Accreditation of Healthcare Organizations (JCAHO) (Rivers et al., 2005).

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3 Chapter Three

Methodology

This study was conducted to assess laboratory technicians' knowledge and practices in PBS preparation, staining, and examination, to evaluate the performance of laboratories in PBS examination, and to identify areas for improvement. The ultimate aim was to enhance the quality of laboratory services and contribute to better healthcare in Palestine. This chapter outlines the methodology used, including the study design, study population, study tools, and analysis plan.

3.1 General Study Scheme

This study utilized a mixed-methods design, combining a survey-based assessment of laboratory technicians' knowledge and practices regarding the PBS test, alongside a performance evaluation of clinical laboratories conducting PBS examination in the West Bank. The study aimed to:

- Assess the knowledge and practices of laboratory technicians regarding the preparation, staining, and examination of PBS.
- Evaluate laboratory performance in PBS examination, identifying areas of improvement for laboratories with suboptimal performance.

The approach was carried out in two parts:

1. A comprehensive assessment of laboratory technicians' knowledge and practice in PBS preparation and examination.

2. Identification of laboratories conducting PBS examinations. This part involved surveying laboratories participating in the EQAS program at the Center for Quality in Medical Laboratories (CQML) at Al-Quds University, which is required for laboratory licensing by the Palestinian Ministry of Health (MOH).

This mixed-methods approach allowed for a detailed understanding of laboratory performance, technician practices, and areas needing improvement, which informed the development of targeted interventions and quality control measures. The details of the general study scheme are shown in Figure 3.1.

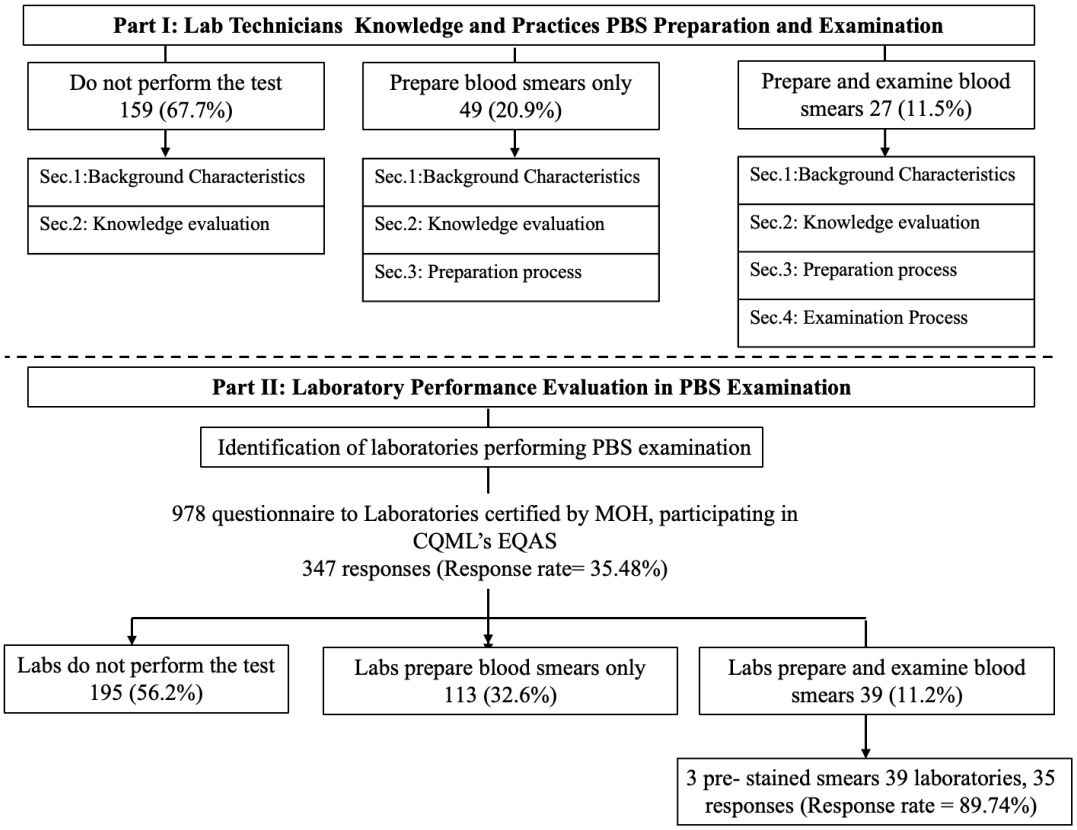

Figure 3. 1: General study scheme.

3.2 Study Population

3.2.1 Assessment of Knowledge and Practice in PBS Preparation, Staining, and Examination Among Laboratory Technicians

The target population for this part of the study included all medical laboratory technicians in Palestine who were licensed by the Palestinian Ministry of Health, regardless of whether they were actively performing PBS testing.

A text message containing an electronic link was created and distributed to all laboratories registered with the CQML, instructing the laboratory technicians to complete the form. The message was also sent to governmental hospitals and primary healthcare centers affiliated with the Ministry of Health. Additionally, the message was circulated to all subcommittees of the Palestinian Medical Technology Association (PMTA).

- **Inclusion criteria**

All medical laboratory technicians in Palestine licensed by the Palestinian Ministry of Health, irrespective of whether they actively perform PBS test.

- **Exclusion criteria**

Unemployed laboratory technicians, technicians not licensed by the Ministry of Health, students, and trainees.

3.2.2 Evaluation of Laboratory Performance in PBS Examination Using Pre-Stained Blood Smears

The study population consisted of laboratories licensed by the MOH and participating in the EQAS program at CQML at Al-Quds University, which prepare and examine PBS. This included private laboratories, hospital laboratories, clinics, health centers, and research facilities. The laboratories were identified through an online screening survey, which was distributed to laboratory managers via text messages containing a link to the "A Survey Form to Identify a Research Sample" (Appendix 3.1). The survey aimed to gather information about the type of laboratory and their involvement in PBS testing.

After two months of data collection, 347 responses were received out of 978, yielding a response rate of 35.5%. Based on the survey responses, laboratories were classified into three groups: (1) laboratories that do not perform PBS testing 195 (56.2%), (2) laboratories that stain PBS slides and send them to another facility for examination 113 (32.6%), and (3) laboratories that perform both staining and examination of PBS within their own facility 39 (11.2%).

Laboratories in group (3), which stain and examine the PBS within their facility, received three pre-stained PBS smears for performance evaluation and were asked to complete the PBS examination form to assess their intralaboratory precision and interlaboratory agreement.

3.3 Data Collection and Study Tools

3.3.1 Assessment of Knowledge and Practice in PBS Preparation, Staining, and Examination Among Laboratory Technicians

To assess laboratory technicians' knowledge and practices regarding PBS preparation, staining, and examination, a questionnaire was constructed using Google Forms (Appendix 3.4).

In addition to collecting demographic data about the participants, the questionnaire was developed based on various literature and hematology books to cover all stages of the blood smear test, from blood sample collection and smear preparation to staining and examination (Adewoyin, 2014; Jones, 2009; K et al., 2022; Theml & Diem, 2011; Vu et al., 2021; Wintrobe & Greer, 2009).

The questionnaire was structured into four main sections to comprehensively assess the participants' background characteristics, knowledge, and laboratory practices related to PBS testing. Section One gathered basic demographic and background characteristics about the participants.

Section Two focused on knowledge evaluation, including sample handling and preparation, the staining process, examination of PBS, and blood cell morphology.

Section Three addressed the procedures for preparing and staining blood smears, targeting laboratory technicians who prepare blood smears, and covering technical details regarding the methods used.

Finally, Section Four, which targeted laboratory technicians who perform PBS examination, focused on the reading of stained PBS, aiming to capture how smears are examined in routine practice. Table 3.1 provides a detailed overview of the questionnaire sections and the topics covered in each section.

Table 3.1: Overview of questionnaire sections for evaluating laboratory technicians' knowledge and practices in PBS testing.

Section number	Topics within the section	No. of Questions
Section One: Demographic and Background Characteristics	A. Background Characteristics	5
Section Two: Knowledge about Blood Smear Preparation, Staining, and Examination	A: Knowledge about Sample handling and preparation	5
	B: Knowledge about PBS Staining Process	6
	C: Knowledge about Blood Smear Examination	8
	D: Knowledge about Blood Cell Morphology	19
Section Three: Procedures for Preparing and Staining Blood Slides in Your Lab	A. Nature of the PBS Examination Work in Your Laboratory	8
	B. Preparation and Staining Blood Slides in Your Laboratory	10
Section Four: Blood Smear Examination in Your Lab	A. Blood Smear Examination in Your Laboratory	7

The questionnaire was designed to be answered based on the specific role of the laboratory technician in the PBS test. Technicians who do not perform the test were instructed to answer only Section 1 (background characteristics) and Section 2 (knowledge evaluation). Technicians who stain the smears only and send them outside for examination were required to complete Sections 1, 2, and 3. Finally, technicians who both stain and examine the PBS within the laboratory were instructed to answer Sections 1, 2, 3, and 4. Table 3.2 illustrates the response mechanism to the questionnaire according to the technician's role in the blood smear test in their workplace.

Table 3.2: Questionnaire sections to be completed according to the technician's role in peripheral blood smear testing.

Role of the technician's in the blood smear test	The sections that need to be answered
Doesn't perform the test	Section 1 + 2
I stain the slides only, and send them outside for examination	Section 1 + 2 + 3
I stain and examine the slides within the laboratory	Section 1 + 2 + 3 + 4

3.3.2 Evaluation of Laboratory Performance in PBS Examination Using Pre-Stained Blood Smears

Pre-stained blood smears were prepared following guidelines based on Clinical and Laboratory Standards Institute (CLSI) recommendations, which were designed for external quality control purposes (Vu et al., 2021). To prepare the blood smears, a blood sample was collected from a volunteer who had undergone a splenectomy, ensuring that all peripheral blood cell lines in the sample were affected and exhibited a moderately abnormal profile (neither completely normal nor extremely abnormal). After selecting the volunteer and obtaining written consent to participate in the study, the blood smear slides were prepared following these steps:

1. Blood sample collection and transport

After obtaining the approval of the donor, a 10 mL whole blood sample was collected from the volunteer at their home using a K2-EDTA tube (Renon) and a 10 mL syringe (Medic) with a needle (Medicare 21G). Immediately after collection, the sample was placed in an ice-packed transport box and delivered to the laboratory for processing within 15 minutes.

2. Preparation of blood smears on glass slides

Upon arrival at the laboratory, the blood was immediately applied to glass slides (Knittel Star Frost slides 76X26 mm). Using an automatic pipette with yellow tips (200 µL, Gilson type), 5 µL of blood were placed on each slide. Another slide of the same type was held at a 45-degree angle to spread the blood evenly. The slides were then left to air-dry for 15 minutes. A total of 200 slides were prepared under the same conditions.

3. Staining of blood smears

All smears were stained using a modified Wright-Giemsa staining kit (BIOGNOST BIO-DIFF KIT), commonly used in hematology labs for rapid blood smear staining. Table 3.3 outlines the composition of the stain kit, and the staining process was performed according to the manufacturer's instructions, as detailed in Table 3.4.

Table 3.3: BIOGNOST BIO-DIFF KIT components.

Reagent name	Description and usage
Bio-Diff 1 Reagent	An alcoholic fixative containing methyl alcohol, used to fix smears as the first step in the staining process
Bio-Diff 2 Reagent	A red staining solution containing eosin Y dye in phosphate buffer, serving as the eosinophilic stain
Bio-Diff 3 Reagent	A blue staining solution containing azure dyes, serving as the basophilic stain
Buffer Tablets (pH 6.8 and pH 7.2)	Used to prepare buffer solutions for rinsing slides during the staining procedure

Table 3. 4: BIOGNOST BIO-DIFF KIT staining procedures for peripheral blood smear.

Step details	Dips / Duration
1. Let the smear dry	
2. Dip the smear into Bio-Diff 1 reagent	5 dips X 1 seconds
3. Decant the excessive reagent from the smear onto filter paper	
4. Dip the smear into Bio-Diff 2 reagent	3 dips X 1 seconds
5. Decant the excessive reagent from the smear onto filter paper	
6. Dip the smear into Bio-Diff 3 reagent	6 dips X 1 seconds
7. Rinse the smear in pH 6.8 buffer solution	Agitation 1 minute
8. Dry the smear	

To ensure uniform staining conditions, a slide holder with a capacity for 24 slides at the same run was used (Figure 3.2).

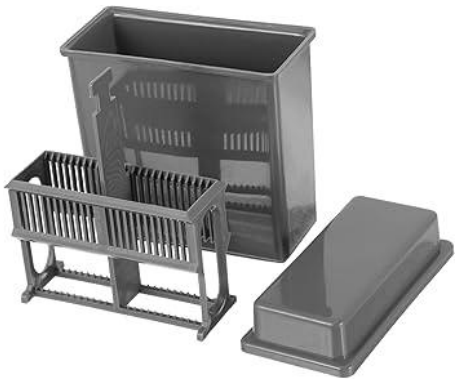

Figure 3. 2: Slides holder with capacity of 24 slides.

4. Application of mounting medium and coverslips

Once the smears were completely dry, a mounting medium (BIMOUNT DPX) and coverslips (BAR-NAOR 24X50 mm) were applied to preserve the stained smears and protect them from environmental factors such as temperature, humidity, and dust. The smears were left in the laboratory for three days to allow the mounting medium to dry completely.

5. Quality assessment of the pre-stained peripheral blood smears

After the mounting medium dried, all smears were reviewed by a hematology specialist to assess the quality of staining and preparation. Random samples of the smears were examined to ensure that the preparation was conducted under optimal conditions and that the staining was homogeneous. Additionally, 9 random smears were distributed to three experts—three smears for each—who were then asked to examine them.

To assess smear homogeneity, a one-way ANOVA was conducted at a significance level of $\alpha = 0.05$ to compare the results provided by the three experts. Each expert evaluated three randomly assigned smears, ensuring that the analysis captured potential variability within and between their assessments (Vives Corrons et al., 2006). The ANOVA results indicated no statistically significant differences between the experts' evaluations in WBC's count estimation (p-value=0.055), differential count of neutrophils (p-value =0.242), lymphocytes (p-value =0.762), monocytes (p-value =0.005), eosinophils (p-value =0.659), basophils (p-value =0.296), band neutrophils (p-value =0.171), RBC's abnormal forms % (p-value =0.622), % of hypochromia (p-value =0.078) and platelets count estimation (p-value =0.106). Hypersegmented neutrophils showed a statistically significant difference between the expert results (p-value =0.015). Additionally, to verify the long-term stability of the smears, one of the experts was asked to retest a smear after five months of distribution, and the expert confirmed that the smear remained stable.

6. Packaging and distribution of the stained smears

Once the quality and homogeneity of the smears was confirmed, each smear was placed in an individual slide mailing box. The smears were then distributed to the 39 participating laboratories. Each participating laboratory received three numbered

smears (Slide 1, Slide 2, Slide 3), along with a PBS Examination Form for recording results (Appendix 3.3).

The PBS Examination Form was developed based on several references, including the hematology textbook *Clinical Hematology and Fundamentals of Hemostasis* by Denise Harmening, 6th edition. Participating laboratories were asked to examine the smears for the estimated white blood cell (WBC) count and differential, evaluate red blood cell (RBC) morphology with identification of abnormal RBC forms and inclusions, and assess the estimated platelet count along with platelet morphology.

3.4 Ethical Considerations

Ethical approval for the study was obtained from the Institutional Review Board (IRB) of Al-Quds University (Appendix 3.6) and the Palestinian Ministry of Health (Appendix 3.7). Informed consent was secured from all participants prior to data collection. For the questionnaire-based components, the first page of the questionnaire included detailed information about the study, its objectives, and the research team, clearly stating that participation was voluntary and that all responses would remain confidential and be used only for scientific research purposes. In the practical phase, all participating laboratories were contacted by phone and provided verbal consent before receiving the pre-stained smears. To ensure participant confidentiality, all collected data were anonymized, securely stored, and accessed only by the research team for the purposes of analysis.

3.5 Data Analysis

3.5.1 Assessment of Knowledge and Practice in Blood Smear Preparation, Staining, and Examination Among Laboratory Technicians

Data were collected through Google Forms and exported to Microsoft Excel, then analyzed using IBM SPSS Statistics version 29.0.0. Descriptive statistics, including frequencies, percentages, means, standard deviations, medians, ranges, and interquartile ranges (IQR), were used as appropriate to summarize technicians' background characteristics, knowledge, and practices related to PBS preparation and examination.

The knowledge section assessed participants' familiarity with PBS preparation, staining, and interpretation using multiple-choice and true/false questions. Each participant's knowledge score (per-person score), converted to a percentage, was calculated using the following formula:

Score = (Participant Number of Correct Answers / Total Possible Answers) × 100.

For knowledge categorization, Bloom's Taxonomy cut-off points were used as a knowledge levels scale to assess participants' knowledge level regarding peripheral blood smear preparation and examination. Previous studies have shown that Bloom's Taxonomy, which categorizes cognitive skills into hierarchical levels ranging from basic recall to complex analysis and evaluation, can be effectively applied in Knowledge, Attitudes, and Practices (KAP) evaluation (Adams, 2015; Ashebir et al., 2022; Zarei et al., 2024). Scores were classified as follows: less than 60% indicates a low knowledge level, 60-80% indicates a medium knowledge level, and above 80% indicates a high knowledge level. These cut-off points are based on commonly used thresholds for knowledge categorization (Tsehay et al., 2024).

Data cleaning was performed to detect outliers before performing the comparative analysis, Tukey's Fences (IQR method), were used. Any data points that lie beyond the calculated fences (which appear outside the whiskers of the boxplot), typically set as $Q1-1.5\times IQR$ for the lower fence and $Q3+1.5\times IQR$ for the upper fence, were considered outliers and eliminated (Letelier et al., 2024).

To compare the knowledge levels between different background characteristics groups (independent variables) based on the total knowledge score (dependent variable), the following statistical tests were used:

- Shapiro-Wilk test to assess the normality of the data.
- Levene's test for homogeneity of variances.
- ANOVA, Mann-Whitney, Kruskal-Wallis tests were used as appropriate to compare knowledge scores between different background characteristics groups.

The significance level was set at $\alpha = 0.05$.

3.5.2 Evaluation of Laboratory Performance in PBS Examination

To evaluate the performance of medical laboratories in the examination of PBS, this study assessed intra-laboratory repeatability (precision), and inter-laboratory results agreement.

To assess intra-laboratory repeatability (precision), each participating laboratory received three pre-stained blood smears derived from the same sample. For numerical parameters, including WBC count estimation, neutrophil differential count, lymphocyte differential count, and platelet count estimation. The repeatability of results across the three smears was evaluated by calculating the coefficient of variation (CV%) for each laboratory for every individual parameter. First, the mean and SD of the three repeated results were calculated, then the CV% were calculated by the formula:

$$CV\% = (SD/mean) * 100$$

where CV% is the coefficient of variation, and SD is the standard deviation.

Since education systems, technical training, and practical exposure differ significantly across countries and even within regions, official bodies like College of American Pathologist (CAP), and National external quality assessment scheme in the United Kingdom (NEQAS) suggest to establish baseline data that reflect local realities. Such data provide a reference point to guide targeted improvements, monitor progress over time. Therefore, the individual CV% results for each laboratory for every individual numeric parameter were described as a CV% range and CV% median to describe the distribution of precision among the laboratories (Lewis, 1995; Vos et al., 2024). To assess the agreement of the laboratory results (inter-laboratory agreement), the three results from each participant were first merged into a single representative value following ISO 13528:2022 (Statistical methods for use in proficiency testing by interlaboratory comparison) and other relevant reference guidelines (ISO13528, 2022; Vives Corrons et al., 2006). Specifically, the mean was used for numerical data, the median for ordinal data, and the mode for nominal data.

Laboratory results agreement was further evaluated as the following:

3.5.2.1 Numerical data

For numerical data, which includes WBCs count estimation, neutrophils and lymphocytes differential, platelets count estimation), the consensus mean and standard deviation of the participating laboratories' results were calculated using the **Robust Algorithm A** method.

The robust mean and standard deviation using Algorithm A, as recommended by ISO 13528:2022, are calculated through an iterative procedure designed to reduce the influence of outliers in interlaboratory or proficiency testing data. The process begins by calculating the initial mean and standard deviation from all participant results. Next, each result is adjusted through a process called standard deviation based **Winsorization**, where any value falling outside the limits calculated as the mean ± 1.5 times the standard deviation is replaced by the corresponding lower or upper limit. A new mean and standard deviation are then computed using these adjusted values. This process is repeated iteratively, updating the mean and standard deviation at each step, until the changes between iterations become very small (converge), typically less than 0.0001. The final values represent the **robust mean (X** *rob***)** and **robust standard deviation (S** *rob***)**, which are less affected by extreme results and provide a reliable measure of group performance.

Once the robust mean and standard deviation are obtained, the **Z-score** for each laboratory result is calculated using the formula:

$$\text{Z-score} = (\mathbf{x}\text{-}\mathbf{X}_{rob}) / \mathbf{S}_{rob}$$

where **x** is the individual laboratory result, **X** *rob* is the robust mean, and **S** *rob* is the robust standard deviation.

Finally, the results of Z-score for each result were interpreted as follows:

- A result with a Z-score (≤ 2 **S** *rob*) is considered acceptable performance.
- A result with ($z > 2$ **S** *rob*) is considered unacceptable performance.

Due to the small reference values and the high frequency of zero results reported by participants, as well as the need for expert judgment in the differential counts of monocytes, eosinophils, basophils, band neutrophils, and hypersegmented neutrophils,

the Algorithm A method did not accurately reflect the actual discrepancies in these types of narrow-range results. Therefore, only descriptive statistics including the range, mean, and median along with the experts' corresponding range, mean, and median, were used.

3.5.2.2 Ordinal and Nominal data

Ordinal and nominal data were used to describe the morphology of normal and abnormal blood cell features. According to ISO 13528, the consensus of participants' results for this type of evaluation is not recommended. The evaluation of morphological features of blood cells requires expert judgment to determine whether the laboratory's results are exactly matched or partially matched, or completely unmatched with the expert judgment.

For ordinal data (Normal, Slight, 1+, 2+, 3+, 4+), the median of the experts' results was considered as the "expert judgment." Then, the median result of each participant was compared to the expert judgment, which included features such as polychromasia, microcytosis, macrocytosis, hypochromia, and abnormal RBC forms. Results that exactly matched the expert judgment were classified as "exactly matched." Results that were one degree higher or lower on the ordinal scale compared to the expert judgment were considered "partially matched." Results that deviated by more than one degree on the ordinal scale from the expert judgment were classified as "completely unmatched."

For nominal data (Seen, Not Seen), including RBC inclusions, the mode of the experts' results was considered the "expert judgment." Then, the mode of each participant's results was compared with the expert judgment. Results that exactly matched the expert judgment were classified as "exactly matched," while results that did not match the expert judgment were classified as "completely unmatched."

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4 Chapter Four

Results

This chapter presents the findings of the study based on the methods outlined in the previous chapter. The results are organized according to the two main parts of the study. The first part presents the assessment of knowledge and practices related to PBS preparation, staining, and examination among laboratory technicians. The second part presents the evaluation of laboratory performance in PBS examination, which was conducted using pre-stained blood smears distributed to participating laboratories.

4.1 Demographic and Background Characteristics of Participating Technicians

In this part of the study, we assessed the knowledge and practices of laboratory technicians using a questionnaire targeting licensed laboratory technicians in Palestine. The questionnaire consisted of four sections. The first section collected background characteristics about the participants and the laboratories in which they work, including laboratory type, educational qualifications, years of experience, and their role in PBS testing.

Based on their involvement in PBS testing, participants were categorized into three groups: those who did not conduct PBS testing in their laboratories (n=159, 67.7%), those who only stained smears and sent them to another entity for examination (n=49, 20.9%), and those who worked in laboratories where they prepared, stained, and examined peripheral blood smears (n=27, 11.5%). Participants in the first category

were asked to complete only the knowledge evaluation section. Those in the second category completed both the knowledge evaluation section and the section on procedures for preparing and staining blood smears. The third group was asked to complete all three relevant sections: the knowledge evaluation, the procedures for preparing and staining blood slides, and the section the examination of stained blood smears.

A total of 235 laboratory technicians participated in the study. In terms of educational qualifications, 169 (71.9%) of the participants held a bachelor's degree, followed by 61 (26.0%) with a master's degree, 4 (1.7%) with a diploma, and 1 (0.4%) with a PhD. Regarding the type of laboratory in which they were employed, 91 participants (38.7%) worked in private laboratories, 82 (34.9%) in hospital laboratories, and 62 (26.4%) in primary health care laboratories.

Regarding years of experience, 85 participants (36.2%) had less than 5 years of experience, 50 (21.3%) had 6–10 years, 37 (15.7%) had 11–15 years, and 63 (26.8%) had more than 15 years of experience. Regarding involvement in PBS testing, 159 participants (67.7%) reported that they do not perform the test, while 49 (20.9%) were responsible for smear preparation only, and 27 (11.5%) performed both smear preparation and examination.

4.2 Assessment of Knowledge in Blood Smear Preparation, Staining, and Examination Among Laboratory Technicians

The knowledge Evaluation section of the questionnaire assessed four areas of knowledge about PBS testing; knowledge about sample handling and preparation, knowledge about staining process, knowledge about smear examination, and knowledge about blood cell morphology.

4.2.1 Knowledge about Sample Handling and Preparation

This section assessed participants' understanding of proper sample handling and preparation techniques for PBS testing. Among the five evaluated items, the highest proportion of correct responses was related to the appropriate anticoagulant, with 231

out of 235 respondents (98.3%) correctly identifying EDTA as the preferred choice. A similarly high percentage (94.9%; 223 participants) correctly recognized the thin smear technique as appropriate for evaluating blood cell morphology and quantity. Knowledge of smear preparation was also strong, with 223 participants (94.4%) correctly indicating that smears should be air dried before applying a fixer.

Slightly lower scores were noted for specific technical steps, such as the correct angle for spreading the smear, which was answered correctly by 208 participants (88.5%), and the appropriate timing of smear preparation, correctly identified by 202 participants (86.0%). Although these items showed slightly lower accuracy, the overall performance still reflects a generally high level of knowledge.

Participants' total scores in this domain ranged from 40% to 100%, with a mean of 92.51% (SD=11.3) and a median of 100% (IQR=20, 80-100). Only 1 participant (0.4%) scored < 60%, indicating low knowledge levels, and 78 (33.2%) indicated medium knowledge, while 156 (66.4%) achieved scores above 80%, indicating high knowledge in this domain.

These findings suggest that most participants possess a solid understanding of essential concepts related to sample handling and PBS preparation. The distribution of knowledge scores about sample handling and preparation is shown in Figure 4.1 (histogram), and Figure 4.2 (boxplot) presents the interquartile range and highlights potential outliers.

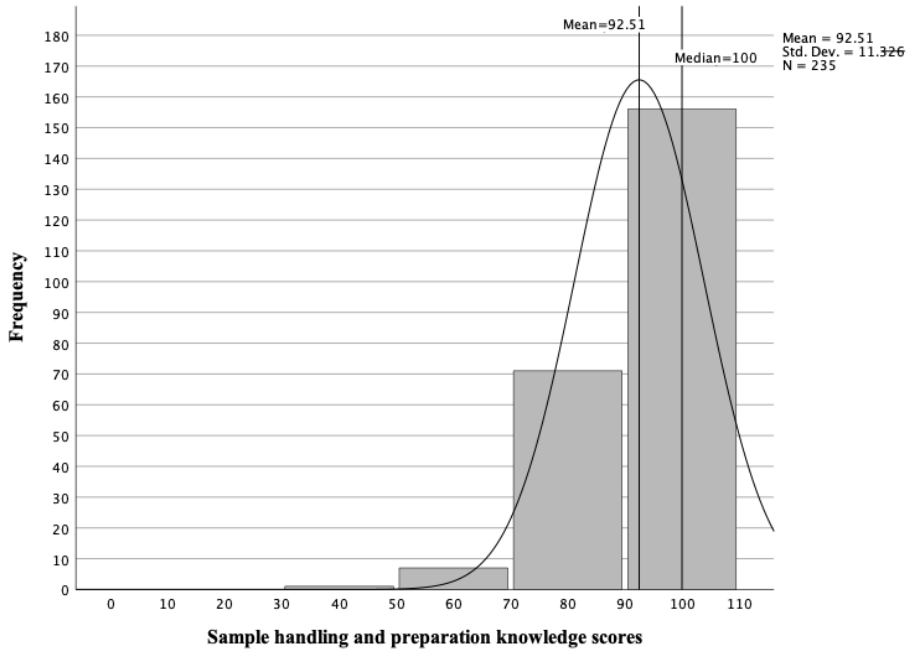

Figure 4.1: Distribution of knowledge scores about sample handling and preparation.

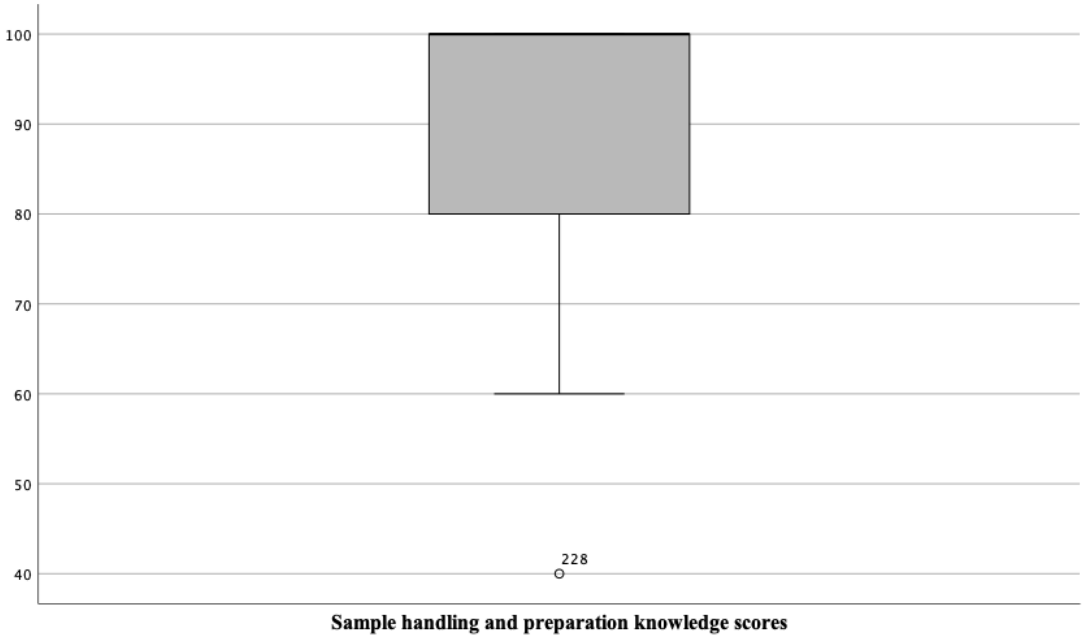

Figure 4.2: Box Plot of knowledge scores about sample handling and preparation.

4.2.2 Knowledge about PBS Staining Process

This section evaluated participants' knowledge about staining procedures involved in PBS preparation. Methylene Blue was identified as a component of the stain by 207 (88.1%) of the participants. Likewise, 171 participants (72.8%) correctly indicated that excess stain should be removed by rinsing with tap water and allowing the smear to dry. In contrast, fewer participants correctly identified Eosin as a key stain component (n = 134; 57.0%), and only 107 participants (45.4%) correctly identified Romanowsky stains as the commonly used stain type for PBS. Knowledge related to fixatives showed the greatest variation: 137 participants (58.3%) correctly selected 100% Methanol, while only 44 (18.7%) selected 100% Ethanol as a fixative agent.

Overall, knowledge scores in this section were lower compared to other domains. Participants' scores ranged from 0% to 100%, with a mean of 56.7% (SD = 21.3) and a median of 50% (IQR=33, 33-66). Out of 235 participants, 123 (52.3%) scored < 60%, indicating low knowledge, and 66 (28.1%) scored between 60% and 80% indicating medium knowledge, while only 46 (19.6%) achieved scores above 80% indicating high knowledge.

These results suggest that while participants demonstrated good knowledge of certain aspects of the staining process, such as identifying individual stain components and post-staining steps, they showed less understanding of stain classification and the various fixatives. This may reflect inconsistencies in laboratory practices related to smear staining.

The distribution of knowledge scores about the staining process is illustrated in Figure 4.3. Additionally, a box plot was constructed to visualize the interquartile range and identify potential outliers in participants' scores (Figure 4.4).

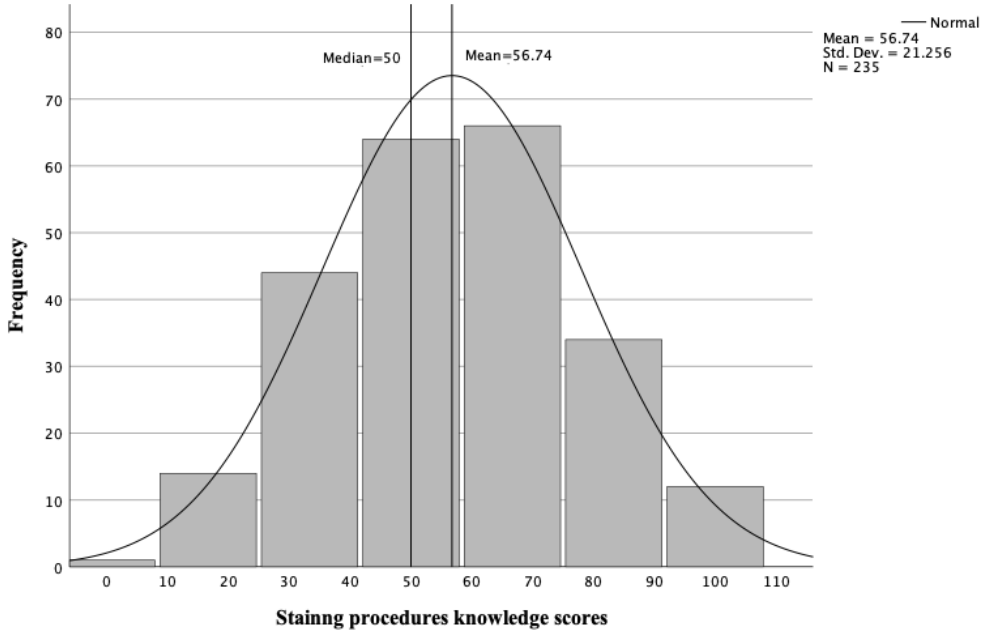

Figure 4.3: Distribution of knowledge scores about staining process.

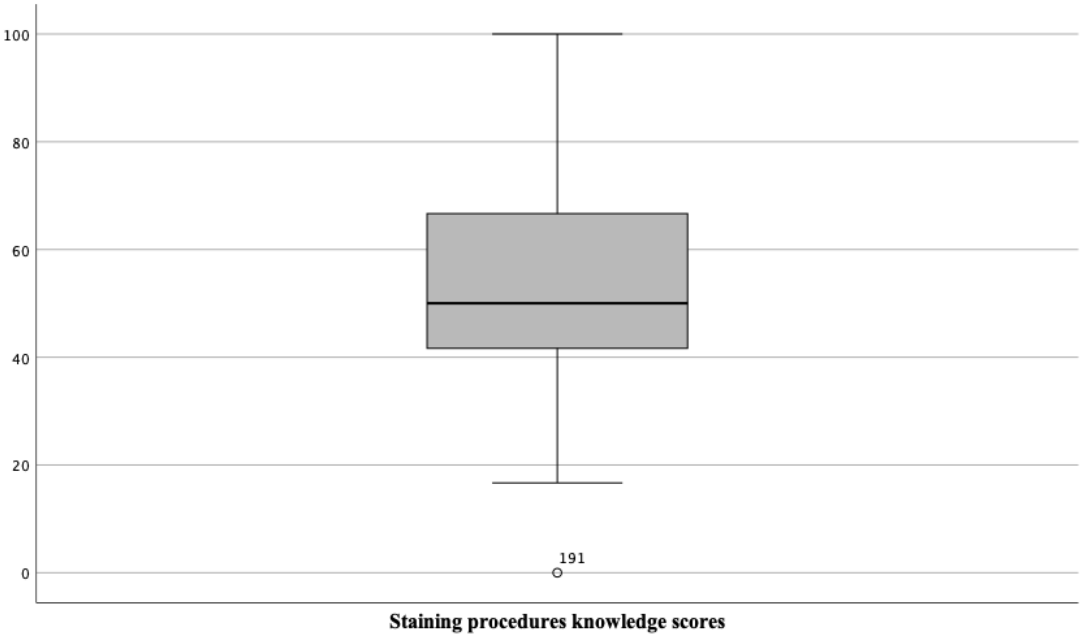

Figure 4.4: Box plot of knowledge scores about staining process.

4.2.3 Knowledge about Blood Smear Examination

This section assessed participants' knowledge of microscopy techniques and interpretive procedures used in PBS examination. Participants demonstrated stronger knowledge in certain areas, particularly in the use of the oil-immersion lens (100x). Specifically, 173 participants (73.6%) correctly identified that the oil-immersion lens is used to evaluate red blood cell morphology (in terms of shape, size, and inclusions), estimate platelet count, and assess platelet morphology. Similarly, 168 participants (71.5%) also identified it as an appropriate lens for performing white WBC differential counts, and 162 participants (68.9%) correctly indicated that 100 WBCs should be counted in a manual differential count. In contrast, lower correct answers were observed for questions involving the selection of lower magnification objectives. Only 76 participants (32.3%) correctly selected the low-power lens (10x) for assessing the general quality of stained smears, and 78 participants (33.2%) correctly identified the high-power lens (40x) as suitable for WBC count estimation. Furthermore, just 123 (52.3%) of the participants correctly answered that 10 microscopic fields are required for WBC estimation. Correct answers were higher for the question regarding the need to correct WBC counts in the presence of NRBC, with 189 participants (80.4%) responding correctly.

Overall, knowledge scores in this domain ranged from 0% to 100%, with a mean of 60.74% (SD = 18.4), a median of 62.5% (IQR=25, 50-75). Out of 235 participants, 89 (37.9%) scored below 60% indicating low knowledge, 117 (49.8%) achieved scores between 60% and 80% indicating medium knowledge, and only 29 (12.3%) scored above 80% indicating high knowledge.

These findings indicate that while participants showed adequate understanding about oil-immersion lens usage, such as differential counts and morphological assessments, there was a noticeable lack of clarity concerning the appropriate use of lower magnification objectives.

The distribution of knowledge scores about blood smear examination is shown in Figure 4.5, while Figure 4.6 (boxplot) was constructed to visualize the interquartile range and detect potential outliers in participants' scores.

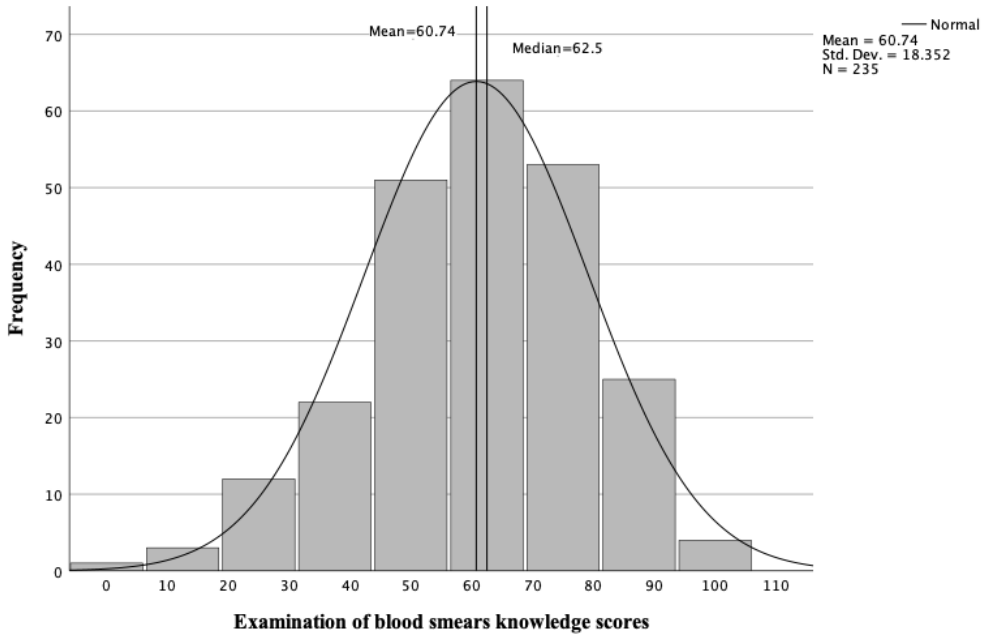

Figure 4.5: Distribution of knowledge scores about blood smear examination.

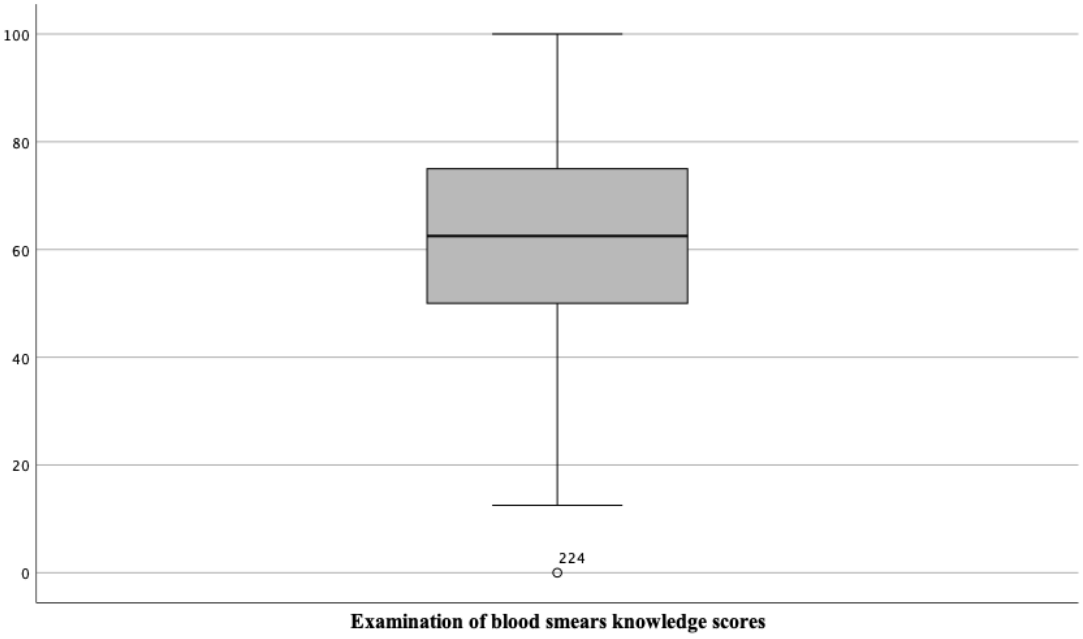

Figure 4.6: Box plot of knowledge scores about blood smear examination.

4.2.4 Knowledge about Blood Cell Morphology

This section evaluated participants' ability to identify various blood cells and morphological features using 19 images. Participants exhibited a good understanding of RBC morphology, with moderate to high correct identification rates for tear drop cells (88.9%), sickle cells (86%), acanthocytes (80%) and normal erythrocytes (80%), and stomatocytes (74.9%). Identification of target cells (71.9%) and reticulocytes (62.1%) was also acceptable. However, nucleated red blood cells (NRBCs) were correctly identified by only 55 participants (23.4%), marking this item as the least recognized RBC-related structure. For RBC inclusions, most participants correctly identified Howell-Jolly bodies (76.6%), while a moderate percentage recognized Cabot rings (62.6%) and basophilic stippling (58.3%).

In the WBC images, the hypersegmented neutrophil was correctly identified by 182 participants (77.4%), representing the strongest result in this category. In contrast, identification rates for other WBC types were lower: neutrophils (60.9%), eosinophils (48.1%), basophils (48.9%), monocytes (40.4%), and band neutrophils (37.4%). Finally, 182 participants (77.4%) correctly identified platelets.

Participants' knowledge scores in this domain ranged from 11% to 100%, with a mean of 64.05% (SD = 21.0), a median of 68.42% (IQR=32, 47-79). Out of 235 participants, 98 (41.7%) scored below 60% indicating low knowledge levels, while 82 participants (34.9%) achieved scores between 60% and 80% indicating medium knowledge, and only 55 participants (23.4%) scored above 80% indicating high knowledge in this domain.

These findings suggest that participants were generally more familiar with common RBC morphologies and inclusions, while identification of NRBCs and some WBC subtypes, particularly immature or less frequently observed cells, was less consistent.

The distribution of knowledge scores about blood cells morphology is shown in Figure 4.7. Additionally, a box plot was constructed to visualize the interquartile range and detect potential outliers in participants' scores (Figure 4.8).

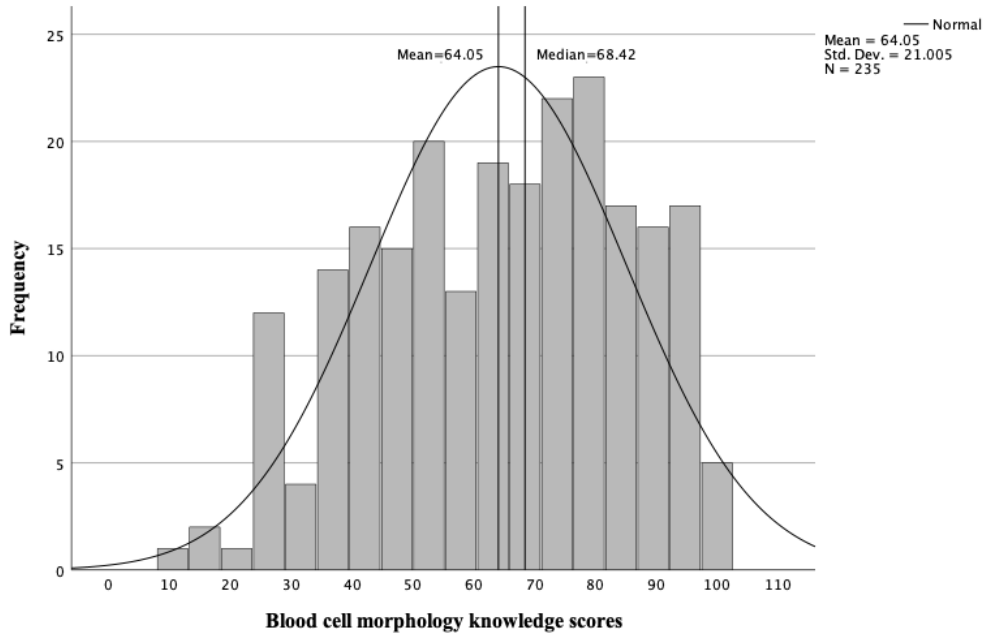

Figure 4.7: Distribution of knowledge scores about blood cell morphology.

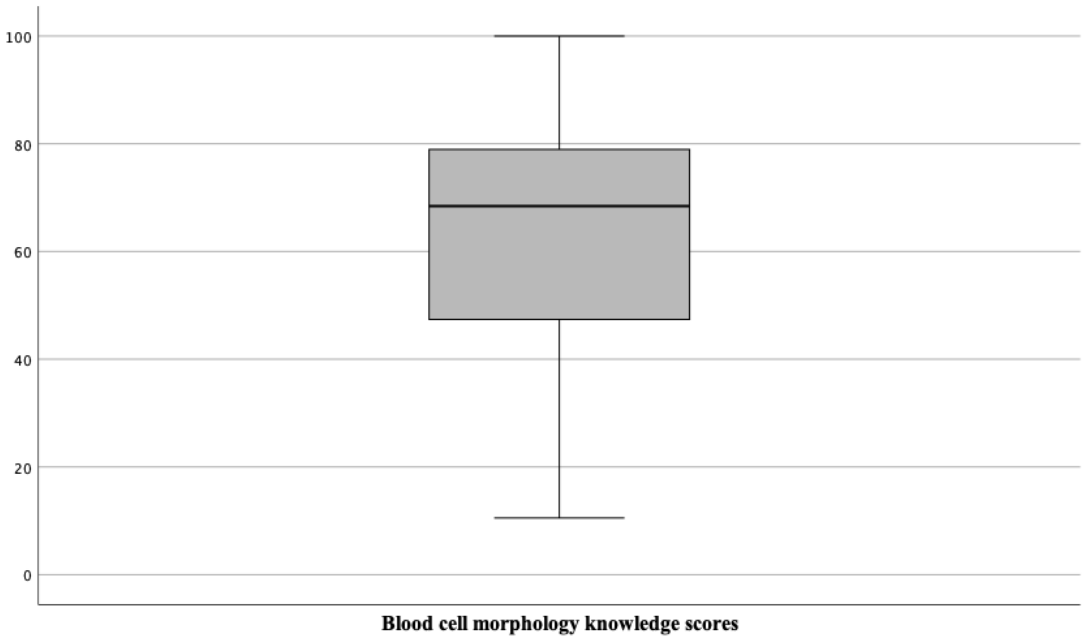

Figure 4.8: Box plot of knowledge scores about blood cells morphology.

4.2.5 Total Knowledge Scores about PBS Preparation, Staining, Examination and Blood Cell Morphology

Participants' total knowledge scores across all sections—sample preparation, staining, examination, and morphology, ranged from 24% to 100%, with a mean of 65.95% (SD = 14.4) and a median of 65.79% (IQR=21, 55.3–76.3). Out of 235 participants, 74 (31.5%) scored < 60% , indicating low knowledge, 119 (50.6%) scored between 60-80%, indicating medium knowledge, and 42 (17.9%) scored > 80%, indicating high knowledge level.

These values indicate low to moderate overall knowledge among the participants. Table 4.1 summarizes the distribution of knowledge categories according to Bloom's cut-off point, while Table 4.2 summarizes the central tendency and dispersion measures, including the mean, median, standard deviation, range, and IQR for each section and the total knowledge scores.

Table 4.1: Summary of knowledge scores for each section, and total knowledge scores based on Bloom's cut-off point.

Section	Low Knowledge (<60%)	Medium Knowledge (60%-80%)	High Knowledge (>80%)
Sample Handling and Preparation	1 (0.4%)	78 (33.2%)	156 (66.4%)
PBS Staining Process	123 (52.3%)	66 (28.1%)	46 (19.6%)
Blood Smear Examination	89 (37.9%)	117 (49.8%)	29 (12.3%)
Blood Cell Morphology	98 (41.7%)	82 (34.9%)	55 (23.4%)
Total Knowledge Score	74 (31.5%)	119 (50.6%)	42 (17.9%)

Table 4.2: Summary of descriptive statistics for knowledge scores of each section, and total knowledge scores.

Section/Subsection	Mean	Median	SD	Range (Min-Max)	IQR
Sample handling and preparation	92.51	100	11.3	60 (40-100)	20
PBS Staining Process	56.74	50	21.3	100 (0-100)	33
Blood Smear Examination	60.74	62.5	18.4	100 (0-100)	25
Blood Cell Morphology	64.05	68.42	21.0	89 (11-100)	32
Total knowledge scores	65.95	65.79	14.4	76 (24-100)	21

The distribution of total knowledge scores is illustrated in Figure 4.9. Additionally, a box plot was constructed to visualize the interquartile range and detect potential outliers in participants' scores (Figure 4.10).

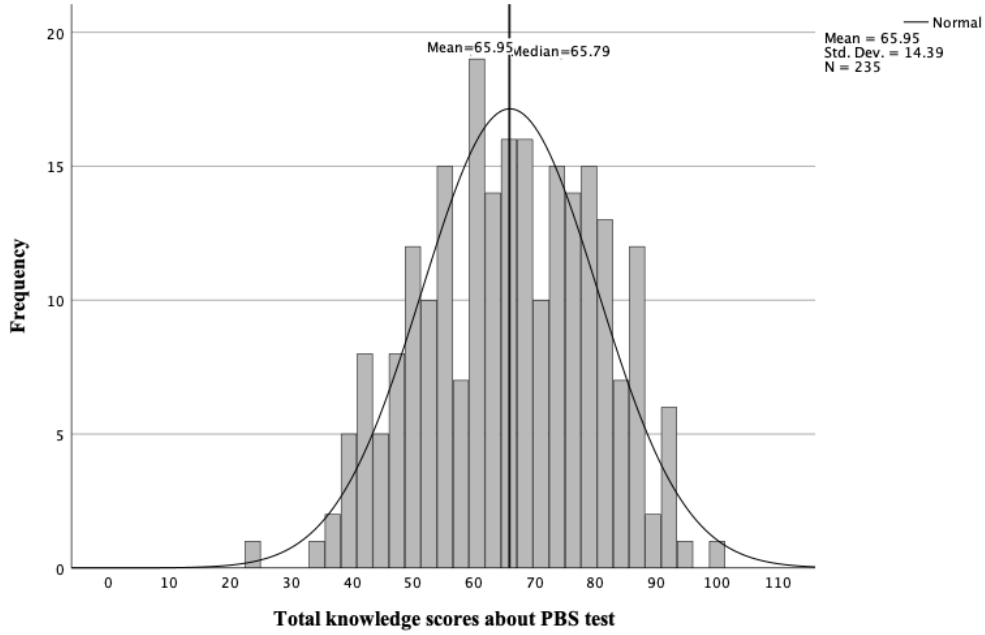

Figure 4.9: Distribution of total knowledge scores about preparation, staining, examination and blood cell morphology.

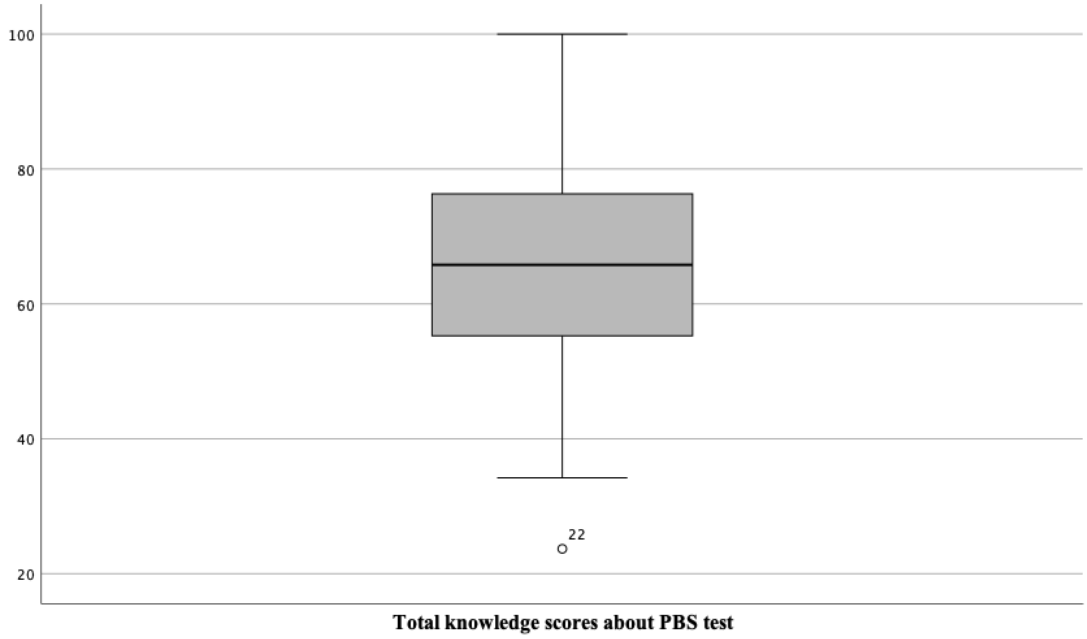

Figure 4.10: Box plot of total knowledge scores about preparation, staining, examination and blood cell morphology.

4.2.6 Analysis of Total Knowledge Scores Based on Background Variables

In this section, statistical tests were used to compare knowledge levels between groups with different background characteristics (independent variables), based on the total knowledge score (dependent variable). These background characteristics included information about the participants and the laboratories in which they work, such as laboratory type, educational qualifications, years of experience, and their role in PBS testing.

4.2.6.1 According to Laboratory Type

The distribution of knowledge levels across different workplace settings is presented using cross-tabulation. Out of 235 participants, 91 (38.7%) were employed in private laboratories, with 25 (27.5%) showing low knowledge, 49 (53.8%) medium knowledge, and 17 (18.7%) high knowledge. In hospitals' laboratories, 82 (34.9%) participants were employed, of whom 23 (28.0%) had low knowledge, 44 (53.7%) medium knowledge, and 15 (18.3%) high knowledge. Finally, 62 (26.4%) participants worked in primary healthcare center laboratories, with 26 (41.9%) showing low knowledge, 26 (41.9%) medium knowledge, and 10 (16.1%) high knowledge

To determine whether the mean knowledge assessment scores for the PBS test differed significantly among laboratory technicians based on the type of laboratory in which they work, we used one-way Analysis of Variance (ANOVA).

The assumptions of normality and homogeneity of variances were evaluated. The Shapiro-Wilk test indicated that the distribution of knowledge scores was normal for all three groups: private laboratories ($p = 0.188$, $n = 91$), hospital laboratories ($p = 0.138$, $n = 81$), and primary healthcare centers ($p = 0.067$, $n = 62$). Levene's test for homogeneity of variances was not significant ($p = 0.230$), confirming the assumption of equal variances between groups.

The ANOVA revealed no statistically significant difference in mean knowledge scores between the three laboratory types, $F(2, 231) = 1.151$, $p = 0.318$. The mean score of participants from private laboratories was 67.1 (SD = 15.4), for hospitals laboratories 66.9 (SD = 13.0), and for primary healthcare laboratories 63.8 (SD = 13.8). The effect size, as measured by Eta-squared ($\eta^2 = 0.010$), indicating a small effect of laboratory type on knowledge score. Table 4.3 summarizes the distribution of knowledge score categories among laboratory technicians according to the type of laboratory in which they work. It also presents the descriptive statistics of the overall knowledge scores for each laboratory type.

Table 4.3: Distribution of knowledge score categories and descriptive statistics according to laboratory type.

Laboratory type	Low knowledge N (%)	Medium knowledge N (%)	High knowledge N (%)	Knowledge score Mean±SD	IQR	P-value
Private	25 (27.5%)	49 (53.8%)	17 (18.7%)	67.1 (±15.4)	24	0.318
Hospital	23 (28.0%)	44 (53.7%)	15 (18.3%)	66.9 (±13.0)	20	
Primary health care	26 (41.9%)	26 (41.9%)	10 (16.1%)	63.8 (±13.8)	21	

4.2.6.2 According to Years of Experience

The distribution of participating technicians based on their years of experience was as the following: < 5 years (n = 85, 36.2%), 6–10 years (n = 50, 21.3%), 11–15 years (n = 37, 15.7%), and > 15 years (n = 63, 26.8%). Among those with < 5 years of experience, 32 (37.6%) had low knowledge, 43 (50.6%) medium knowledge, and 10 (11.8%) high knowledge. Those in the 6–10 years group, 16 (32%) had low knowledge, 28 (56%) medium knowledge, and 6 (12%) high knowledge. Among those with 11–15 years of experience, 13 (35.1%) had low knowledge, 16 (43.2%) medium, and 8 (21.6%) high. Participants with > 15 years of experience, 13 (20.6%) showed low knowledge, 32 (50.8%) medium, and 18 (28.6%) high.

To examine whether there is a statistically significant difference in the knowledge assessment scores for PBS tests according to years of experience, normality and homogeneity of variance assumptions were tested. The dependent variable was the total knowledge score, and the independent variable was years of experience, categorized into four groups: < 5 years, 6–10 years, 11–15 years, and > 15 years.

The assumption of normality was assessed using the Shapiro-Wilk test, the data were normally distributed for all groups: < 5 years ($p = 0.210$), 6–10 years ($p = 0.374$), 11–15 years ($p = 0.634$), and > 15 years ($p = 0.113$). The assumption of homogeneity of variances was tested using Levene's test and was met ($p = 0.301$).

The mean knowledge score was 63.6 (SD = 13.5) for participants with < 5 years of experience, 64.5 (SD = 13.5) for 6–10 years, 65.7 (SD = 16.5) for 11–15 years, and 71.1 (SD = 13.2) for those with > 15 years of experience.

A one-way ANOVA revealed a statistically significant difference in knowledge scores between groups, F (3, 230) = 3.882, p = 0.010, with a small effect size ($\eta^2 = 0.048$).

Post hoc using Tukey's test showed a significant difference between participants with < 5 years of experience and those with > 15 years of experience (mean difference = -7.534, p = 0.007). No other pair comparisons were statistically significant. Table 4.4 summarizes the distribution of knowledge score categories among laboratory technicians according to their years of experience. It also presents the descriptive statistics of the overall knowledge scores for each experience group.

Table 4.4: Distribution of knowledge score categories and descriptive statistics according to years of experience.

Experience (years)	Low knowledge N (%)	Medium knowledge N (%)	High knowledge N (%)	Knowledge score Mean±SD	IQR	p-value
< 5	32 (37.6%)	43 (50.6%)	10 (11.8%)	63.7 (±13.5)	21	0.010
6 - 10	16 (32.0%)	28 (56.0%)	6 (12.0%)	64.5 (±13.5)	19	
11 - 15	13 (35.1%)	16 (43.2%)	8 (21.6%)	65.7 (±16.5)	28	
> 15	13 (20.6%)	32 (50.8%)	18 (28.6%)	71.1 (±13.2)	21	

4.2.6.3 According to the Laboratory Technician Role in PBS Test

A total of 159 participants (67.7%) reported that they do not perform PBS testing, 57 (35.8%) of them had low knowledge, 83 (52.2%) medium knowledge, and 19 (11.9%) high knowledge. Additionally, 49 participants (20.9%) were involved in smear preparation and staining only, 10 (20.4%) of them had low knowledge, 27 (55.1%) medium knowledge, and 12 (24.5%) high knowledge. Those involved in smear preparation and examination 27 (11.5%), 7 (25.9%) of them had low knowledge, 9 (33.3%) medium knowledge, and 11 (40.7%) high knowledge.

To compare knowledge assessment scores among the participants based on their role in PBS testing, assumptions of normality were assessed using the Shapiro-Wilk test. The results showed a violation of normality in the group of participants who do not perform the PBS test (Shapiro-Wilk p = 0.007), whereas the other two groups demonstrated normality (smear preparation only: p = 0.117; preparation and examination: p = 0.138). Accordingly, nonparametric test was used.

The Kruskal-Wallis H test was used to determine whether there were statistically significant differences in knowledge scores between the groups. The results showed a statistically significant difference across groups: H (2) = 9.467, p = 0.009.

Participants who do not perform the PBS test (n = 159) had a mean score of 64.1 (SD = 13.0) and a median of 65.8. Those who prepare smears only (n = 48) had a higher mean score of 70.0 (SD = 14.3) and a median of 68.4. Participants who perform both smear preparation and examination (n = 27) showed the highest mean score of 71.0 (SD = 18.0) and a median of 76.3. Table 4.5 presents the distribution of knowledge score categories among laboratory technicians according to their role in the peripheral blood smear (PBS) test. The table also includes descriptive statistics for the knowledge score across different roles.

Table 4.5: Comparison of knowledge score categories and descriptive statistics according to laboratory technician role in PBS test.

Role in PBS test	Low knowledge N (%)	Medium knowledge N (%)	High knowledge N (%)	Knowledge score Mean±SD	Median	IQR	p-value
Do not perform	57 (35.8%)	83 (52.2%)	19 (11.9%)	64.1 (±13.0)	65.8	18	0.009
Staining only	10 (20.4%)	27 (55.1%)	12 (24.5%)	70.0 (±14.3)	68.4	20	
Staining and Examination	7 (25.9%)	9 (33.3%)	11 (40.7%)	71.0 (±18.0)	76.3	26	

4.2.6.4 According to Educational Qualification

Based on the educational qualifications of the participants, 4 had a diploma, 169 had a bachelor's degree, 61 had a master's degree, and 1 participant had a PhD. To ensure more balanced group sizes, diploma and bachelor's degree holders were combined into a single group labeled "graduates" (n=173; 73.6%), while those with a master's or PhD degree were grouped as "postgraduates" (n=62; 26.4%). Among the graduates, 59 (34.1%) had low knowledge, 90 (52.0%) medium knowledge, 24 (13.9%) high knowledge, while 15 (24.2%) of the postgraduate group had low knowledge, 29 (46.8%) had medium knowledge, 18 (29.0%) had high knowledge.

The data were tested for normality by Shapiro–Wilk test, the test revealed that it is not normally distributed (p = 0.015 for graduates and p = 0.042 for postgraduates). To compare the total knowledge scores of laboratory technicians based on their

educational qualifications, a Mann–Whitney U test was conducted. The dependent variable was the total knowledge score, and the independent variable was educational qualification, categorized as graduates and postgraduates.

The graduates group (n = 173) had a mean score of 64.6 (SD = 13.3) and a median of 65.8, while the postgraduates group (n = 62) had a mean score of 70.41 (SD = 15.7) and a median of 73.7. The Mann–Whitney U test showed a statistically significant difference between the two groups (U = 3973, z = -2.872, p = 0.004), indicating that postgraduates had significantly higher knowledge scores compared to graduates. Table 4.6 summarizes the distribution of knowledge score categories among laboratory technicians according to their highest educational qualification. The table also presents descriptive statistics for the knowledge score within each qualification group.

Table 4.6: Comparison of knowledge score categories according to laboratory technicians' educational qualification.

Education	Low knowledge N (%)	Medium knowledge N (%)	High knowledge N (%)	Knowledge score Mean±SD	Median	IQR	p-value
Graduate	59 (34.1%)	90 (52.0%)	24 (13.9%)	64.6 (±13.3)	65.8	21	0.004
Post graduate	15 (24.2%)	29 (46.8%)	18 (29.0%)	70.4 (±15.7)	73.7	21	

4.3 Laboratory Technicians Practice Assessment in PBS Preparation and Examination

This section of the questionnaire was completed by 76 participants, 49 (64.5%) of them were involved in smear preparation only, and 27 (35.5%) performed both smear preparation and examination.

4.3.1 General Characteristics of PBS Test in Participants' Laboratories

Among the 76 laboratory technicians, only 33 (43.4%) reported receiving special training specifically in PBS testing.

The frequency of conducting PBS tests varied across laboratories. PBS testing was performed on a daily basis in 16 laboratories (21.1%), weekly in 25 laboratories (32.9%), monthly in 30 laboratories (39.5%), and annually in 5 laboratories (6.6%).

When asked about the conditions under which PBS testing is performed, 61.3% of participants reported performing the test based on a physician's request, while 23.6% indicated it is used to confirm abnormal results of other tests, and 15.1% conducted the test in response to warning flags generated by automated hematology analyzers.

Only 16 participants (21.1%) reported that their laboratories participate in an EQAS for PBS testing. However, 54 (71.1%) participants reported routinely evaluating the quality of PBS smears prepared in their laboratories.

Regarding specimen preservation practices, only 20 participants (26.3%) reported using a mounting medium to preserve smears when the PBS is not examined immediately. In cases where smears were transported outside the laboratory for examination, 17 (22.4%) participants indicated a transport time of less than 2 hours, 11 (14.5%) reported 2–4 hours, 2 (2.6%) 4–6 hours, and another 17 (22.4%) reported a transport time of more than 6 hours. Only 6 participants (7.9%) indicated using a special box with ice for transport.

4.3.2 Preparation and Staining Blood Smears

Out of 76 participants, 72 participants (94.7%) reported using a manual method for PBS preparation, while 4 (5.3%) indicated using an automated method.

Regarding sample collection, 74 (97.4%) participants used EDTA as the anticoagulant of choice, while only 1 participant (1.3%) used citrate, and another 1 (1.3%) used fresh capillary blood without anticoagulant. Among those using EDTA, 24 (31.6%) reported using K2-EDTA, 51 (67.1%) reported using K3-EDTA, and 1 laboratory (1.3%) reported using Na-EDTA.

The drying time after blood spreading on the glass slide varied from 2 to 30 minutes, with a mean of 14.9 minutes (±9.2), and a median of 15 minutes.

Regarding the fixative agents used, 54 participants (71.1%) reported using absolute methanol, 13 (17.1%) reported using 90% ethanol, and 9 (11.8%) reported using

absolute ethanol. The duration of fixation ranged from 2 to 30 minutes, with a mean of 6.9 minutes (±6.3) and a median of 5 minutes. In terms of staining procedures, 58 participants (76.3%) reported using Giemsa stain, 12 (15.8%) used Wright stain, and 6 (7.9%) used Diff-Quick stain. The reported staining duration ranged from 2 to 40 minutes, with an average of 12.08 minutes (±8.9) and a median of 13 minutes. Table 4.7 summarizes the staining processes of PBS among the participants' laboratories.

Table 4.7: A summary of staining processes of PBS among the participants' laboratories.

Staining process	Time range (min)	Median (min)	Mean (min)	SD (min)
Drying duration	(2 – 30) min	15	14.9	9.2
Fixation duration	(2 – 30) min	5	6.9	6.3
Staining duration	(2 – 40) min	13	12.08	8.9

4.3.3 Blood Smear Examination in Participants' Laboratory

This section presents the current practices reported by 27 laboratory professionals for examining white blood cells (WBCs), red blood cells (RBCs), and platelets in peripheral blood smears.

In the examination of WBCs, 37.0% (n=10) of participants reported using the high-power objective lens (40×) to estimate the WBC count, while the majority, 63.0% (n=17), used the oil-immersion objective lens (100×).

The number of microscopic fields assessed to estimate the WBC count ranged from 2 to 50 fields, with an average of 14.3 fields (±11) and a median of 10 fields.

For performing the WBC differential count, 7.4% (n=2) used the low-power objective lens (10×), 33.3% (n = 9) used the high-power objective lens (40×), and 59.3% (n = 16) used the oil-immersion objective lens (100×). The number of WBCs counted during the differential ranged from 5 to 200 cells, with an average of 84.96 cells (±59.48) and a median of 100 cells.

Regarding the correction of WBC count in the presence of nucleated red blood cells (NRBCs), participants indicated they perform corrections when NRBCs exceed

thresholds ranging from more than 5 to more than 20 cells, with an average threshold of 10.9 cells, a median of 10 cells, and a standard deviation of 5.4.

For the examination of RBC morphology, 3.7% (n = 1) of participants used the low-power objective lens (10×), 25.9% (n = 7) used the high-power objective lens (40×), and the majority, 70.4% (n = 19), used the oil-immersion objective lens (100×) to analyze RBC shape, size, inclusions, and the presence of abnormal forms.

In assessing platelets, 22.2% (n = 6) of participants used the high-power objective lens (40×), while 77.8% (n = 21) relied on the oil-immersion objective lens (100×) to evaluate platelet size, morphology, and estimate the platelet count.

4.4 Evaluation of Laboratory Performance in PBS Examination

This section evaluates the performance of the participating laboratories in PBS examination through three key measures. First, intra-laboratory repeatability (precision) is assessed by calculating the CV% across the three repeated results from each laboratory. Second, inter-laboratory precision is evaluated by comparing numerical results across laboratories using the overall CV%. Third, assessment of agreement between the laboratory's result using the Z-score method. For the evaluation of morphological features, the level of agreement between laboratory findings and expert assessments is also examined.

4.4.1 Distribution of Laboratories in the West Bank Based on Their Role in the PBS Test

The responding laboratories included private laboratories 191 (55.0%), 87 (45.5%) of which do not perform the test, 78 (40.8%) only prepare smears, and 26 (13.6%) both prepare and examine smears. Hospitals' laboratories accounted for 29 (8.4%) of the responses, 8 (27.6%) of them not performing the test, 13 (44.8%) only preparing smears, and 8 (27.6%) both preparing and examining smears. Primary health care laboratories represented 87 (25.1%) of the responses, 83 (95.4%) of them do not perform the test, 4 (4.6%) only prepare smears, and none perform examination. Private medical center laboratories accounted for 40 (11.5%) of the responses, 17

(42.5%) of them not performing the test, 18 (45%) only preparing smears, and 5 (12.5%) both preparing and examining smears. A total of 117 blood smears were distributed to 39 laboratories, three for each. Table 4.8 summarizes the involvement of various laboratory types based on their roles in PBS testing.

Table 4.8: Involvement of different laboratory types according to their roles in PBS testing.

Laboratory type	Total N (%)	Do not perform the test	Smear preparation only	Preparation & Examination
Private lab	191 (55.0%)	87 (45.5%)	78 (40.8%)	26 (13.6%)
Hospitals lab	29 (8.4%)	8 (27.6%)	13 (44.8%)	8 (27.6%)
Primary healthcare lab	87 (25.1%)	83 (95.4%)	4 (4.6%)	0 (0.0%)
Medical centers lab	40 (11.5%)	17 (42.5%)	18 (45%)	5 (12.5%)

4.4.2 Evaluation of Laboratory Performance in Blood Cell Count Estimation and Differential

In this section, we evaluated the performance of the participating laboratories in blood cell count estimation, including manual WBC and platelet counts, as well as manual WBC differential counts, specifically for neutrophils and lymphocytes. For each parameter, the individual CV% for the three repeated results for each laboratory was calculated and presented as a CV% range (intra-laboratory CV%) and median. For the agreement of the results between the laboratories, Z-score analysis was used.

For the WBC count estimation, the individual laboratories' CV% ranged from 0.5% to 73.6%, with a median CV% of 28.1%. This indicates a wide variability in intra-laboratory precision among participants, with several laboratories showing high levels of imprecision. For the consensus result of the laboratories, the Robust mean of Algorithm A (robust mean) was 6.93, and the Robust standard deviation of Algorithm A (robust SD) was 2.03 (CV% = 29.3). The Z-score analysis showed that 33 out of 35 laboratories had acceptable performance (94.3%), while 2 laboratories (5.7%) had unacceptable performance. Despite notable intra-laboratory variation, most laboratories demonstrated acceptable inter-laboratory Z-score, indicating general agreement with the consensus value.

The individual laboratory CV% for **Neutrophils differential count** ranged from 0.0% to 21.3%, with a median CV% of 10.0%. This reflects good precision among laboratories, with low variability in reported neutrophil differential count. For the consensus result of the laboratories, the robust mean was 58.6, and the SD was 6.03(CV% = 10.3). The Z-score analysis showed that 33 out of 35 laboratories (94.3%) had acceptable performance, while 2 laboratories (5.7%) had unacceptable performance. The high rate of acceptable Z-scores, along with low CV%, suggests reliable inter- and intra-laboratory performance for neutrophil counts.

For **Lymphocytes differential count**, the individual laboratory CV% ranged from 0% to 60.1%, with a median CV% of 19.9%. This suggests a moderate level of variability in lymphocyte differential count, with some laboratories showing marked imprecision. For the consensus result of the laboratories, the robust mean for was 27.6, and the SD was 6.05(CV% = 21.9%). The Z-score analysis showed that 34 out of 35 laboratories (97.1%) had acceptable performance, while 1 laboratory (2.9%) had unacceptable performance. Although the CV% was relatively high, the majority of laboratories aligned well with the consensus, indicating satisfactory agreement.

For the **Platelet count estimation**, the individual CV% ranged from 0.4% to 100.3%, with a median CV% of 31%. This wide range reflects considerable inconsistency in intra-laboratory precision, with some laboratories showing extreme variability. For the consensus result of the laboratories, the robust mean was 564.9, and the SD was 283 (CV%=50.1%). The Z-score analysis showed that 35 out of 35 laboratories (100%) had acceptable performance, with no laboratories having unacceptable performance. Despite the high inter-laboratory variation (as indicated by the large CV%), all laboratories remained within acceptable limits based on Z-score analysis, suggesting general agreement in platelet estimation at the group level.Table 4.9 summarizes the performance evaluation of laboratories in various parameters, based on both intra-laboratory precision (CV%) and inter-laboratory agreement (Z-score analysis). For each parameter, the CV% range, median, and Z-score results are presented to assess the consistency and agreement of laboratory performance in estimating different blood components.

Table 4.9: Summary of intra-laboratory precision and inter-laboratory agreement for blood cell count estimation and differential count.

Parameter	Intra-lab CV% median (range)	Robust Mean	Robust SD	CV%	Acceptable performance	Unacceptable performance
WBC count estimation	28.1 (0.5 - 73.6)	6.93	2.03	29.3	33 (94.3%)	2 (5.7%)
Neutrophil diff. count	10.0 (0.0 - 21.3)	58.6	6.03	10.3	33 (94.3%)	2 (5.7%)
Lymphocyte diff. count	19.9 (0.0 – 60.1)	27.6	6.05	21.9	34 (97.1%)	1 (2.9%)
Platelet count estimation	31 (0.4 – 100.3)	564.9	283	50.1	35 (100%)	0 (0.0 %)

Due to the small reference values and the high frequency of zero results reported by participants, as well as the need for expert judgment in the differential counts of monocytes, eosinophils, basophils, band neutrophils, and hypersegmented neutrophils, the Algorithm A method did not accurately reflect the actual discrepancies in these types of narrow-range results. Therefore, only descriptive statistics, including the range, mean, and median, along with the experts' corresponding range, mean, and median, were used.

For **Monocyte differential counts**, the expert results ranged from 0.67% to 4.67% with a mean of 2.55% and a median of 2.33%. In contrast, laboratory values showed a wider range (0.0% to 10.0%), with a higher mean of 3.76% and median of 4.0%, suggesting a tendency among participants to overestimate monocyte percentages. This discrepancy suggests limited consistency with expert judgment and may reflect challenges in accurately identifying or distinguishing monocytes during manual differential counts.

In the case of **Eosinophils differential count**, expert values were tightly clustered (range: 4.0% to 5.33%), with a mean of 4.55% and median of 4.33%. However, laboratories reported a broader range (0.3% to 8.3%) and a lower mean (3.63%) and median (3.33%). These findings highlight variability in eosinophil detection and a potential trend toward underestimation in some laboratories, which may be due to morphological misclassification.

Basophil differential counts reported by experts ranged from 0.0% to 1.33%, with a mean of 0.55% and median of 0.33%, reflecting the typically low physiological levels of these cells. Participating laboratories, however, showed more variability, with counts ranging from 0.0% to 8.3%, a mean of 1.47%, and a median of 1.0%. This

marked variability suggests frequent overestimation by laboratories, possibly due to difficulties in accurately identifying basophils, which are morphologically subtle and often misclassified.

For **band neutrophils differential count**, both experts and laboratories reported almost similar ranges (0.0% to 4.67% for experts and 0.3% to 5.0% for laboratories), and comparable mean and median values. This close agreement indicates a relatively high level of consistency and reliability among laboratories in identifying band neutrophils.

Hypersegmented neutrophils showed the largest discrepancy. Experts reported a range of (0% to 22%), a mean of 8.88%, and a median of 4.66%. In contrast, laboratories reported a narrower and lower range (0.0% to 14.67%), with a substantially lower mean of 2.5% and a median of 0.83%. This discrepancy may reflect under recognition of hypersegmentation by laboratory staff, which can have clinical implications, and may reflect limited training or experience in identifying this morphological abnormality. Table 4.10 summarizes the descriptive statistics of WBC differential counts reported by experts and participating laboratories.

Table 4.10: Summary of WBC differential count data reported by experts and participating laboratories.

WBC differential	Expert/Lab	Range (%)	Mean (%)	Median (%)
Monocytes	**Expert**	0.67 - 4.67	2.55	2.33
	Lab	0.0 - 10	3.76	4.0
Eosinophils	**Expert**	4.0 - 5.33	4.55	4.33
	Lab	0.3 - 8.3	3.63	3.33
Basophils	**Expert**	0.0 - 1.33	0.55	0.33
	Lab	0.0 - 8.3	1.47	1.0
Band Neutrophils	**Expert**	0.0 - 4.67	2.0	1.33
	Lab	0.3 - 5.0	1.72	1.33
Hypersegmented Neutrophils	**Expert**	0.0 - 22	8.88	4.66
	Lab	0.0 - 14.67	2.5	0.83

4.4.3 Evaluation of Laboratory Performance in Blood Cells Morphology

The evaluation of RBC morphology includes the parameters of polychromasia, microcytosis, macrocytosis, and hypochromia. The laboratories' results were categorized according to their degree of agreement with the experts' results.

The expert judgment for **polychromasia** was “normal.” Among the participating laboratories, 23 (65.7%) reported results that exactly matched the expert judgment, indicating a generally good level of agreement; however, 9 laboratories (25.7%) only partially matched, and 3 (8.6%) had missing values.

For **microcytosis**, the expert judgment was “1+.” A lower level of agreement was observed for this parameter, with only 4 laboratories (11.4%) exactly matching the expert evaluation, while 13 (37.1%) partially matched and 15 (42.9%) were completely unmatched, suggesting noticeable variability in the assessment. Three laboratories (8.6%) had missing values.

Regarding **macrocytosis**, the expert judgment was “normal.” A total of 19 laboratories (54.3%) exactly matched the expert rating, while 1 (2.9%) partially matched and 13 (37.2%) were completely unmatched. Two laboratories (5.7%) had missing responses.

For **hypochromia**, the expert judgment was “1+.” Exactly matched results were reported by 12 (34.3%) laboratories, 14 (40.0%) were partially matched, and 6 (17.1%) were completely unmatched. Three laboratories (8.6%) had missing values.

Overall, the results indicate varying levels of agreement across the different RBC morphology parameters, with microcytosis and macrocytosis showing higher discrepancies compared to polychromasia. Table 4.11 summarizes the evaluation results of participants' performance regarding RBC morphology evaluation parameters.

Table 4.11: Results summary of laboratory performance evaluation in RBC morphology assessment.

Parameter	Expert Judgment	Exactly Matched (n, %)	Partially Matched (n, %)	Completely Unmatched (n, %)	Missing (n, %)
Polychromasia	Normal	23 (65.7%)	9 (25.7%)	0 (0.0%)	3 (8.6%)
Microcytosis	1+	4 (11.4%)	13 (37.1%)	15 (42.9%)	3 (8.6%)
Macrocytosis	Normal	19 (54.3%)	1 (2.9%)	13 (37.2%)	2 (5.7%)
Hypochromia	1+	12 (34.3%)	14 (40.0%)	6 (17.1%)	3 (8.6%)

The evaluation of laboratory performance in identifying abnormal RBC forms showed considerable variability across different cell types. The laboratories' results were categorized according to their degree of agreement with the experts' results.

For **spherocytes**, where the expert judgment was "1+," only 3 (8.6%) laboratories exactly matched the expert evaluation, 7(20%) partially matched, while the majority 23(65.7%) were completely unmatched, indicating a significant challenge in accurately identifying this feature.

In the case of **tear drop cells**, judged as "normal" by the experts, 11 (31.4%) laboratories exactly matched the expert judgment, while 19 (54.3%) partially matched, showing some uncertainty among participants in identifying this abnormality. Regarding **target cells**, which were rated as "1+" by experts, 17 (48.6%) laboratories exactly matched the expert judgment. However, 8 (22.8%) were partially matched and 8 (22.8%) were completely unmatched, indicating moderate variability in recognition accuracy for this abnormality.

For **bite cells**, experts reported them as "Not seen," and 21 1 (60%) laboratories correctly reported them as not seen. However, 7 (20%) partially matched and 5 (14.3%) were completely unmatched, suggesting some false positive identifications. **Stomatocytes**, also judged "Not seen" by the experts, were accurately reported by 24 (68.6%) laboratories, though 6 (17.1%) partially matched and 3 (8.6%) were completely unmatched.

Schistocytes were also judged as "Not seen." The majority of laboratories 21 (60%), exactly matched this, 8 (22.9%) partially matched, and 4 (11.5%) were completely unmatched. For **sickle cells**, the expert judgment was "Not seen," and 25 (71.4%) laboratories were exactly matched, with no laboratories completely mismatching the expert judgment, reflecting high reliability in recognizing this abnormality.

In the evaluation of **acanthocytes**, also reported as "Not seen" by the experts, 23 (65.7%) laboratories exactly matched, while only 1 lab (2.9%) completely mismatched, showing good agreement.

Conversely, **ovalocytes**, judged as "slight" by experts, had low agreement: only 4 (11.4%) laboratories exactly matched, while 19 (54.3%) were completely unmatched, indicating difficulty in ovalocyte identification.

Lastly, for **echinocytes**, judged as normal, 9 (25.7%) laboratories exactly matched the expert judgment, but a significant proportion, 23 (65.8%) laboratories, partially matched, reflecting inconsistency in interpreting this morphology. Table 4.12 summarizes the evaluation results of participants' performance in identifying RBC abnormal forms.

Table 4.12: Results summary of laboratory performance in assessing RBC abnormal forms.

Abnormal RBC Form	Expert Judgment	Exact Match (n,%)	Partial Match (n,%)	Unmatched (n,%)	Missing (n,%)
Spherocytes	1+	3 (8.6%)	7 (20%)	23 (65.7%)	2 (5.7%)
Tear drops	Normal	11 (31.4%)	19 (54.3%)	3 (8.6%)	2 (5.7%)
Target cells	1+	17 (48.6%)	8 (22.8%)	8 (22.8%)	2 (5.7%)
Bite cells	Not seen	21 (60%)	7 (20%)	5 (14.3%)	2 (5.7%)
Stomatocytes	Not seen	24 (68.6%)	6 (17.1%)	3 (8.6%)	2 (5.7%)
Schistocytes	Not seen	21 (60%)	8 (22.9%)	4 (11.5%)	2 (5.7%)
Sickle cells	Not seen	25 (71.4%)	8 (22.9%)	0 (0%)	2 (5.7%)
Acanthocytes	Not seen	23 (65.7%)	9 (25.7%)	1 (2.9%)	2 (5.7%)
Ovalocytes	Slight	4 (11.4%)	10 (28.6%)	19 (54.3%)	2 (5.7%)
Echinocytes	Normal	9 (25.7%)	23 (65.8%)	3 (2.9%)	2 (5.7%)

The evaluation of laboratory performance in identifying RBC inclusions showed varying degrees of agreement with expert judgment. For Howell-Jolly bodies, only 12 (34.3%) laboratories exactly matched the experts' judgment of "seen," while the majority, 21 (60.0%) laboratories, failed to identify them, indicating a significant gap in the detection of this inclusion.

For the remaining inclusions—Basophilic stippling, Pappenheimer bodies, Cabot rings, Heinz bodies, and Hemoglobin crystals—the expert judgment was "not seen." Most laboratories exhibited high consistency with these assessments, with exact matches reported in 85.7% of cases for Basophilic stippling, Pappenheimer bodies, Heinz bodies, and Hemoglobin crystals, and 88.6% for Cabot rings. A small proportion of laboratories (ranging from 5.7% to 8.6%) reported completely unmatched results, suggesting potential over-identification or misinterpretation. Overall, these findings indicate a strong performance in ruling out non-present inclusions but highlight challenges in correctly identifying present inclusions like Howell-Jolly bodies. Table 4.13 summarizes the evaluation results of participants' performance in identifying RBC Inclusions.

Table 4.13: Results summary of laboratory performance in assessing RBC inclusions.

RBC Inclusion	Expert Judgment	Exact Match (n, %)	Unmatched (n, %)	Missing Values (n, %)
Howell-Jolly	Seen	12 (34.3%)	21 (60.0%)	2 (5.7%)
Basophilic stippling	Not seen	30 (85.7%)	3 (8.6%)	2 (5.7%)
Pappenheimer bodies	Not seen	30 (85.7%)	3 (8.6%)	2 (5.7%)
Cabot ring	Not seen	31 (88.6%)	2 (5.7%)	2 (5.7%)
Heinz bodies	Not seen	30 (85.7%)	3 (8.6%)	2 (5.7%)
Hemoglobin crystals	Not seen	30 (85.7%)	3 (8.6%)	2 (5.7%)

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5 Chapter Five

Discussion, Conclusions, Limitations and Recommendations

This study was conducted to comprehensively evaluate laboratory technicians' practices and knowledge about PBS testing, alongside evaluation of laboratory performance in PBS examination, with the aim of enhancing diagnostic reliability and contributing to healthcare improvement in Palestine. This chapter discusses and interprets the main findings of the study. Additionally, it presents the study's conclusions, outlines its limitations, and suggests recommendations.

5.1 Discussion

This study aimed to evaluate the quality of peripheral blood smear (PBS) testing in medical laboratories across the West Bank through a comprehensive assessment of laboratory technicians' knowledge and practices regarding PBS preparation, staining, and examination, as well as evaluation of laboratory performance in PBS examination.

The findings revealed significant variability in both technician knowledge, practices, and laboratory performance. While knowledge related to sample handling and smear preparation was relatively high, marked deficiencies were observed in areas requiring greater interpretive and technical expertise, such as the staining process, smear

examination, and blood cell morphology identification. In addition, notable inconsistencies in intra-laboratory precision and inter-laboratory agreement pointed to a lack of standardization in PBS examination practices across laboratories. These findings underscore the urgent need to implement a national EQAS to promote consistency, accuracy, and diagnostic reliability in PBS testing.

Based on the results, this discussion will explore how the findings align with the study's hypotheses. The first hypothesis addressed the knowledge of laboratory technicians, proposing that significant deficiencies would exist and would be influenced by demographic and background characteristics. The second hypothesis proposed that laboratory performance in PBS examination would exhibit significant variability in both intra-laboratory and inter-laboratory measures.

5.1.1 Characteristics and Distribution of Participating Technicians and Laboratories

5.1.1.1 Demographic and Background Characteristics of Laboratory Technicians

The background characteristics of the 235 participating laboratory technicians highlighted several patterns relevant to PBS testing capacity in Palestine. In terms of education, the majority had a bachelor's degree (71.9%), followed by a smaller proportion with a master's degree (26.0%), and minimal representation from diploma (1.7%) and PhD holders (0.4%). This suggests a relatively uniform distribution of academic profiles among laboratory technicians.

Regarding the type of laboratory where participants work, 38.7% were employed in private laboratories, 34.9% in hospital laboratories, and 26.4% in primary healthcare laboratories. These figures suggest that PBS services are primarily concentrated in the private and hospital sectors, where access to equipment, supervision, and standardized protocols may vary significantly.

Experience levels also varied: 36.2% had < 5 years of experience, 21.3% had 6–10 years, 15.7% had 11–15 years, and 26.8% had > 15 years. Despite this range, involvement in the full PBS testing process was limited. Most technicians (67.7%) did not perform PBS testing, 20.9% were involved in smear preparation only, and only

11.5% carried out both preparation and examination. This limited engagement in the complete diagnostic process may restrict the development of practical and interpretive skills and contribute to variability in PBS knowledge, practices, and overall laboratory performance.

5.1.2 Distribution and Implementation of PBS Testing in Laboratories

The distribution of laboratories according to their roles in PBS testing across the West Bank reveals a fragmented and inconsistently implemented system. Private laboratories represented the majority of respondents (55.0%), yet only 13.6% performed both smear preparation and examination. Similarly, in hospital laboratories (8.4%), only 27.6% were involved in the complete PBS process. The situation was more limited in the primary healthcare sector, which accounted for 25.1% of responses, where 95.4% of laboratories did not perform PBS testing at all, and only 4.59% were involved in smear preparation without examination. A similar pattern was observed in private medical centers (11.5%), where 42.5% did not conduct PBS testing, 45% prepared smears only, and only 12.5% completed both steps. This uneven implementation reflects a significant structural limitation in the delivery of PBS testing services, restricting technicians' exposure to the full practical process, particularly morphological evaluation. This limitation may restrict the development of interpretive and practical skills, which ultimately affect the accuracy and reliability of PBS-based diagnostics.

These findings are consistent with previous studies that point to a global shift away from manual smear reviews in favor of automation (Adewoyin, 2014; Chase et al., 2023). However, despite this trend, the diagnostic value of PBS remains essential, particularly in identifying hematologic abnormalities that automated analyzers may fail to detect (Gulati et al., 2013). The current study also found wide variation in the frequency and indications for performing PBS testing. Only 21.1% of laboratories reported conducting PBS test on a daily basis, while most performed them weekly (32.9%) or monthly (39.5%). Moreover, testing was primarily triggered by physician request (61.3%), with less emphasis on using PBS as a tool to confirm flagged or abnormal results from automated hematology analyzers. This aligns with findings by Leung et al (Leung et al., 2021), who reported that 70% of blood smears were not reviewed by laboratory staff unless requested by a physician.

Together, these findings highlight a fundamental structural challenge in the current laboratory system. Limited and inconsistent exposure of technicians to the full PBS testing process may reduce their opportunities for regular practice and skill reinforcement, particularly for technicians not involved in smear examination, which may ultimately compromise diagnostic quality and delay patient care. There is an urgent need for a more integrated, standardized, and accessible approach to PBS testing across all laboratory sectors in Palestine.

5.1.3 Knowledge Assessment in PBS Preparation, Staining, and Examination

One of the primary objectives of this study was to assess the level of knowledge among laboratory technicians regarding PBS preparation, staining, and examination. The results revealed substantial variability across different aspects of the PBS test, supporting the first alternative hypothesis (H1,1), which proposed that laboratory technicians exhibit significant deficiencies in PBS-related knowledge.

5.1.3.1 Knowledge Assessment Findings

In the domain of **sample handling and preparation**, participants performed well. A total of 98.3% correctly identified EDTA as the anticoagulant of choice, and 94.9% recognized the thin smear technique as suitable for evaluating cell morphology. Similarly, 94.4% correctly mentioned that smears should be air dried before fixation. Even for more technical aspects, such as the correct angle for spreading the blood sample over the glass slide (88.5%) and timing (86.0%), the knowledge level remained relatively high. Only 0.4% scored below 60%, while 66.4% showed high knowledge in this domain. These findings suggest that concepts related to sample handling and preparation are well understood, likely due to the routine and standardized nature of these tasks in many laboratories.

By contrast, the knowledge about the PBS **staining process** was notably lower. Although 88.1% correctly identified methylene blue as a stain component and 72.8% understood the proper post-staining rinse and drying steps, only 57.0% identified eosin as one of the stain components, and just 45.4% recognized that Romanowsky stains are the type used in PBS staining. Moreover, 58.3% correctly identified 100% methanol as one of the proper fixative agents, while only 18.7% identified 100% ethanol as a fixative agent. In total, 52.3% scored below 60%, indicating low

knowledge in this domain. These findings indicate inconsistency in theoretical understanding of the staining process, suggesting a lack of standardized practice or training.

In the section assessing knowledge about **smear examination**, the results were similarly mixed. High percentages of participants correctly identified key uses of the oil-immersion objective lens: 73.6% linked it to RBC morphology assessment, and 71.5% linked it to WBC differentials. Furthermore, 68.9% recognized that 100 WBCs should be counted in a manual differential. However, knowledge was lower regarding low and high-power magnification objectives: only 32.3% correctly identified the 10× lens for smear quality check, and 33.2% selected the 40× objective for WBC estimation. Overall, 37.9% scored below 60%, while only 12.3% demonstrated high knowledge. These results reflect the limited practical experience in PBS examination approaches among many technicians, especially those not directly responsible for examining the smears.

Regarding **blood cell morphology**, participants demonstrated moderate familiarity with common RBC forms such as tear drop cells (88.9%), sickle cells (86.0%), acanthocytes (80.0%), and stomatocytes (74.9%). However, correct identification rates were lower for diagnostically significant but less frequently observed structures: NRBCs were correctly recognized by only 23.4%, and WBC recognition was variable, band neutrophils (37.4%), monocytes (40.4%), basophils (48.9%). Platelets, in contrast, were correctly identified by 77.4%. This pattern suggests that while technicians are able to recognize common or classical morphological patterns, they are less familiar to identify atypical or abnormal forms that are often critical for diagnosis. However, this observed score may overestimate the actual capabilities of laboratory technicians, this is because the assessment part of the study relied on image-based evaluations that predominantly contained cells with normal morphology, with only a few abnormal forms.

When aggregated across all domains, total knowledge scores revealed that 31.5% had low overall knowledge (<60%), 50.6% fell in the range (60–80%) and had medium knowledge, and only 17.9% achieved high knowledge (>80%). The total knowledge mean score was 65.95%. These findings confirm a significant gap in comprehensive

PBS knowledge among laboratory technicians, especially in procedural and interpretive aspects of the PBS test.

The relatively strong knowledge in sample preparation contrasts with a weaker understanding of staining principles and cell morphology, areas that are more complex and interpretive in nature. These deficiencies may result from inconsistent training, limited exposure to full smear preparation and examination process, and the absence of continuing professional development programs focused on PBS test.

5.1.3.2 Influence of Demographic and Background Characteristics on Knowledge Scores

To better understand the factors influencing laboratory technicians' knowledge about PBS testing, total knowledge scores were analyzed in relation to key background variables. These included laboratory type, years of experience, technician role in PBS testing, and educational qualification. The findings provide important insights into both institutional and personal factors that contribute to variability in PBS-related knowledge, further supporting the second alternative hypothesis (H1,2), which anticipated that background characteristics would significantly influence knowledge levels.

According to the **type of laboratory** in which the participants work, the one-way ANOVA test revealed that there is no statistically significant difference in knowledge scores among technicians working in private laboratories, hospitals, and primary healthcare centers laboratories ($p = 0.318$). While the mean scores were slightly higher in private (67.1%) and hospitals laboratories (66.9%) compared to primary healthcare centers (63.8%), these differences were not statistically meaningful. This suggests that knowledge gaps are not restricted to a specific laboratory type but may reflect broader systemic issues across all institutional settings. Despite minor variations in the proportion of technicians with high knowledge (18.7%) in private laboratories, 18.3% in hospitals laboratories, and 16.1% in primary health care laboratories, the overall pattern suggests comparatively low levels of understanding. These findings confirm the need for uniform training and quality assurance protocols regardless of laboratory type, highlighting that improving PBS competency requires national-level standardization, not just institutional improvement.

Years of experience emerged as a significant factor affecting knowledge scores. The one-way ANOVA test revealed a statistically significant difference among the four experience groups ($p = 0.010$). Notably, participants with > 15 years of experience had the highest mean score (71.1%), compared to those with < 5 years (63.6%). Post hoc testing confirmed a significant difference between these two groups (mean difference = -7.534, $p = 0.007$).

These results indicate that experience plays a vital role in knowledge development, likely due to cumulative exposure and greater professional maturity. However, it is important to note that even among the most experienced group, only 28.6% scored in the high knowledge category, suggesting that experience alone does not guarantee comprehensive PBS knowledge, particularly in the absence of continuing education and assessments.

The **technician's role in PBS testing** was also significantly associated with knowledge outcomes. The Kruskal-Wallis H test revealed a statistically significant difference in total knowledge scores across the three groups ($p = 0.009$). Participants who performed both smear preparation and examination had the highest mean knowledge score (71.0%), followed by those who prepared smears only (70.0%), and those not involved in PBS testing (64.1%).

Interestingly, 40.7% of those who involved in PBS preparation and examination achieved high knowledge scores, compared to only 11.9% among those with no involvement. This reinforces the practical importance of involvement in PBS testing to improve technician's competency. Technicians who are routinely responsible for preparing and examining smears are more likely to gain knowledge. The data support calls for expanding technicians' involvement in the entire testing process, especially in facilities that only prepare the smears.

Finally, **educational qualification** was significantly associated with PBS knowledge levels. Participants with higher educational qualifications (master's or PhD) had a higher mean knowledge score (70.4%) compared to lower level (diploma and bachelor's holders; score = 64.6%). The difference was statistically significant according to the Mann–Whitney U test ($p = 0.004$).

Among postgraduates, 29.0% demonstrated high knowledge, nearly double the percentage observed among graduates (13.9%). This finding confirms the positive impact of advanced education on technical and analytical competencies in laboratory practice. It also highlights the potential benefit of integrating higher level hematology training within continuing education programs to present persistent knowledge gaps.

Taken together, the results of the knowledge assessment provide clear evidence supporting the first alternative hypothesis (H1,1), which proposed that laboratory technicians in the West Bank exhibit significant deficiencies in PBS-related knowledge, and that these deficiencies are influenced by their background characteristics. These findings justify the rejection of the first null hypothesis (H0,1) and confirm that knowledge gaps are both present and unevenly distributed among technicians. This variability highlights the need for educational interventions, targeted continuing professional development, and standardized guidelines to ensure that all laboratory personnel possess the necessary knowledge to perform PBS testing accurately and reliably.

5.1.4 Assessment of Laboratory Technicians' Practice in PBS Preparation and Examination

Another central objective of this study was to evaluate the practical aspects of how laboratory technicians in Palestine perform PBS preparation and examination. The findings reflect a generally inconsistent approach to PBS practices, with notable variation in training, preparation techniques, staining protocols, and examination approaches. These discrepancies align with and further support the first alternative hypothesis (H1,1), which proposed that significant variability exists in PBS-related practices among laboratory technicians.

5.1.4.1 General Characteristics of PBS Test in Laboratories

Among the 76 technicians who completed the practices section, fewer than half (43.4%) reported having received formal training specific to PBS testing. This limited exposure to structured instruction may be a contributing factor to inconsistent practices and underlines a systemic gap in professional development. The irregularity in testing frequency, conducted daily in only 21.1% of laboratories, and monthly or

less in nearly half (46.1%)—further suggests that PBS is not yet a routine, standardized component of laboratory services across the region.

Notably, only 16 participants (21.1%) reported participating in any EQAS program for PBS. While 71.1% claimed to routinely assess smear quality internally, the absence of external quality standard limits the objectivity and comparability of these evaluations. The reliance on physician request (61.3%) as the primary reason for performing PBS also points to a reactive, rather than standardized, diagnostic approach.

Storage and transport conditions for PBS smears varied significantly. Only 26.3% used mounting media to preserve smears when not examined immediately, and just 7.9% reported using a special box with ice during transportation. Extended transport times—more than 4 hours in 24.9% of cases—raise concerns about the preservation of smear quality and the potential degradation of cellular morphology. These findings highlight practical barriers that can compromise the diagnostic utility of PBS, especially in decentralized or resource-limited entities.

5.1.4.2 Practices in Preparation and Staining of Blood Smears

Preparation techniques also demonstrated considerable inconsistency. The majority (94.7%) of participants relied on manual methods, which can introduce variability depending on the technician's skill and consistency. Despite that the majority (97.4%) of participants using EDTA as an anticoagulant of choice, K3-EDTA (67.1%) was more common than K2-EDTA (31.6%), and this is significant because K2-EDTA is typically advised for hematological analysis because of its less impact on cell morphology (Vu et al., 2021). The reported drying times for blood smears varied widely (2–30 minutes), as did fixation times (2–30 minutes), and staining durations (2–40 minutes). Such variability reflects a lack of standardized protocols, which is concerning given the known impact of these parameters on stain quality and cellular detail. Although 71.1% of participants used absolute methanol as a fixative agent, others used 90% ethanol, which is less effective and may produce water artifacts within the RBC. The CLSI recommends using 100% ethanol because it is safer and less toxic than 100% methanol (Vu et al., 2021).

The staining method also varied, with Giemsa stain used by 76.3% of participants, Wright stain (15.8%) and Diff-Quick (7.9%). Differences in the drying, fixation and duration of staining, even when using the same stain type, further illustrate the absence of a standardized staining protocol across laboratories. These inconsistencies are likely to affect the quality of the blood smear and result in variability in laboratory performance.

5.1.4.3 Practices in Blood Smear Examination

Among the 27 technicians who reported performing smear examinations, there was considerable variation in microscopy practices. A majority used the oil-immersion lens (100×) to estimate WBC counts (63.0%) and perform differential counts (59.3%), despite standard recommendations favoring the high-power (40×) objective for WBC estimation. This misalignment suggests either a lack of awareness of standardized examination protocols or training limitations (Jones, 2009).

Further inconsistencies were identified in examination approaches. The majority of technicians (63.0%) used the oil-immersion objective (100×) to estimate white blood cell (WBC) counts, which is not aligned with guidelines that recommend using the high-power (40×) objective. Additionally, the number of WBCs counted for differential analysis varied widely, ranging from 5 to 200 cells, instead of following the recommended count of 100 cells (Jones, 2009).

Practices related to RBC morphology and platelet assessment also showed inconsistencies in microscopic magnification usage. While over 70% of technicians used 100× oil-immersion objectives, about 30% relied on 40× lens, which lacks the resolution necessary to assess fine morphological details. The use of suboptimal magnification may compromise the ability to detect fine RBC abnormalities, platelet granularity, or size variations, and therefore limits the diagnostic utility of the smear evaluation (Jones, 2009).

These findings indicate a significant discrepancy between recommended and actual practices, especially in laboratories where technicians perform the full examination process. The lack of standardization and deviation from the recommended guidelines could increase the probability of diagnostic inaccuracy.

Taken together, the assessment of laboratory practices in PBS preparation, staining, and examination reveals significant procedural inconsistencies across medical laboratories in Palestine. These deviations from standard guidelines reflect gaps in adherence to established protocols, particularly in smear preparation, staining techniques, and microscopy usage. The findings also show that such inconsistencies are closely linked to technicians' roles and training, with more consistent practices observed among those actively involved in both preparation and examination.

These findings provide strong support for the first alternative hypothesis (H1,1), which proposed that laboratory technicians exhibit significant variability in PBS-related practices. Consequently, the observed procedural variability justifies the rejection of the first null hypothesis (H0,1) with respect to practice, confirming that such inconsistencies are present across technicians. This highlights the urgent need for comprehensive and standardized training programs, national practice guidelines, and ongoing quality monitoring to ensure consistent and reliable PBS testing across laboratories in Palestine.

5.1.5 Evaluation of Laboratory Performance in PBS Examination

A core objective of this study was to assess the performance of medical laboratories in the West Bank in PBS examinations, focusing on both quantitative estimations and morphological assessments. This evaluation includes intra-laboratory results repeatability (precision) using CV%, and how much the results agree between laboratories using Z-scores obtained from the consensus mean, and the degree of agreement with expert judgment in morphological features. While most laboratories demonstrated acceptable Z-scores for various parameters, a deeper analysis reveals that these apparently favorable results may be misleading due to high variability within the data set. A total of 117 smears were distributed to 39 laboratories (three per laboratory) for performance evaluation

5.1.5.1 Quantitative Performance: A Closer Look at Z-scores and Variability

The Z-score analysis showed that most laboratories fell within the acceptable range for WBC count estimation (94.3%), neutrophil differential (94.3%), lymphocyte differential (97.1%), and platelet estimation (100%). At first glance, these results

suggest a generally high level of agreement between the laboratories. However, these findings must be interpreted with caution.

Z-scores are calculated relative to the **robust mean and standard deviation of the participants' results**. In this study, these parameters were markedly inflated due to high dispersion of the results, especially in the following:

- WBC count estimation: Robust mean= 6.93, SD = 2.03, CV% = 29.3
- Lymphocyte differential count: Robust mean= 27.6, SD = 6.05, CV% = 21.9
- Platelet estimation: Robust mean= 564.9, SD = 283, CV% = 50.1

These elevated standard deviation values statistically widened the acceptable range for Z-scores, allowing laboratories with imprecise or suboptimal results to still fall within the acceptable performance zone. In fact, the consensus means and SD created a broad benchmark that may not accurately reflect true analytical accuracy. Therefore, while the Z-scores appear favorable, they may in fact reflect high data dispersion rather than good proficiency.

These findings highlight the limitations of relying solely on Z-scores in external quality assessments and emphasize the importance of complementing them with descriptive statistics to evaluate the distribution of the data. This aligns with the recommendations of ISO 13528 (ISO13528, 2022).

5.1.5.2 Discrepancies in Differential Counts of WBC Subtypes

Further evidence of inconsistency was found in the comparison of laboratory-reported differential counts to expert-reviewed values. For less common or morphologically subtle WBC subtypes such as monocytes, eosinophils, and basophils, participants tended to either overestimate (e.g., basophils, mean = 1.47% vs. expert mean = 0.55%) or underestimate (e.g., eosinophils, mean = 3.63% vs. expert mean = 4.55%) the true counts.

These discrepancies may reflect challenges in accurately identifying cell types that appear in low numbers or have subtle morphological features, especially in manual differential counts. Such misclassifications are likely influenced by limited exposure

to these cell types, inadequate training, or lack of reference materials. The overestimation of basophils may result from confusion with other dark-staining cells or misinterpretation of poorly stained slides. Conversely, underestimation of eosinophils could indicate difficulty in recognizing their characteristic bilobed nuclei and granules under suboptimal staining or magnification conditions. These findings raise concerns about the diagnostic reliability of differential counts for rare WBCs and emphasize the need for enhanced training in morphology recognition.

5.1.5.3 Performance in RBC Morphology and Abnormal Forms

The evaluation of RBC morphology and abnormal forms revealed substantial inconsistencies in laboratory performance when compared to expert judgment. While some features were identified with moderate accuracy, many others were misclassified or overestimated, particularly in clinically significant or morphologically subtle findings.

Among the general morphology parameters, polychromasia showed the highest agreement (exactly matched, 65.7%) with the expert judgment "normal." However, this result may be misleading. Since polychromatocytes are normally present in low numbers (up to 2%) and have a subtle feature, some participants may have reported "normal" without thoroughly examining the smear. To accurately assess this parameter, a sample containing an increased number of polychromatocytes would be more appropriate, as it would better challenge participants' ability to detect and interpret these few cells.

Microcytosis and macrocytosis demonstrated marked variability. Only 11.4% of laboratories matched the expert assessment of microcytosis (rated 1+), with 42.9% completely unmatched. Macrocytosis showed slightly better agreement (54.3%), yet 37.2% of laboratories reported completely mismatched results. For hypochromia, a key feature in anemia diagnosis, only 34.3% of laboratories matched expert evaluation, with 17.1% providing completely discordant results.

These inconsistencies likely arise from reliance on visual estimation without the support of structured grading systems or validated reference scales. This could decrease reproducibility and add subjectivity to evaluations.

Performance in identifying abnormal RBC shapes was even more variable:

- Spherocytes were correctly identified by only 8.6% of laboratories, while 65.7% were completely unmatched. Given that spherocytosis is a hallmark of certain hemolytic anemias, this low accuracy is diagnostically concerning.

- Tear drop cells, judged as normal by experts, were only correctly reported by 31.4% of laboratories. Over half (54.3%) only partially matched the expert judgment, reflecting uncertainty and potential confusion in recognizing this form. Misidentification could lead to misdiagnosis of marrow disorders such as myelofibrosis.

- Target cells, rated as 1+ by experts, showed moderate agreement, with 48.6% exact matches and 22.8% unmatched. Accurate identification is essential as these cells may indicate conditions such as liver disease or hemoglobinopathies.

Interestingly, laboratories performed better when identifying the absence of abnormal forms. For forms judged as "not seen" by experts, such as bite cells, schistocytes, sickle cells, stomatocytes, and acanthocytes, most laboratories accurately reported their absence. For example, 71.4% correctly reported sickle cells as absent, and 65.7% did so for acanthocytes. This suggests that technicians are more confident in excluding abnormalities than confirming their presence, likely due to greater exposure to normal smears and limited experience with pathological forms.

However, even in the absence of reporting, some abnormalities posed challenges. For instance:

- Bite cells, also judged as "not seen," were incorrectly identified by 14.3% of laboratories, implying false-positive results that could lead to inappropriate clinical suspicion for oxidative hemolysis (e.g., G6PD deficiency).

- Ovalocytes, judged as "slight" by experts, had one of the lowest match rates, only 11.4% matched exactly, while 54.3% were completely unmatched. This suggests difficulty in recognizing these cell forms, which may appear in hereditary elliptocytosis or megaloblastic anemia.

The identification of RBC inclusions further reflected performance gaps. The most pronounced deficiency was seen with Howell-Jolly bodies. Despite being present in the expert-reviewed smears, only 34.3% of laboratories correctly identified them, while 60% failed to detect them altogether. This finding is clinically important, as Howell-Jolly bodies are indicative of splenic dysfunction or post-splenectomy status. Their misidentification highlights a serious limitation in morphology-based diagnostic accuracy.

In contrast, laboratories showed relatively high performance in recognizing the absence of rare inclusions. For example, 88.6% of laboratories accurately reported Cabot ring as not seen, and over 85% did the same for basophilic stippling, Heinz bodies, Pappenheimer bodies, and hemoglobin crystals. This indicates a general tendency toward conservative reporting and greater reliability in ruling out uncommon features likely due to lack of familiarity or fear of overestimation.

As a result, these findings reveal a critical weakness in interpretive practices related to RBC morphology and abnormal forms. Laboratories are generally more accurate in confirming the absence of abnormalities than in detecting their presence, a pattern that suggests insufficient training, limited exposure to pathological smears, and inadequate use of reference materials. These gaps can compromise diagnostic accuracy and delay appropriate clinical decision-making. Improving performance in this area will require focused efforts on training, calibration, and the use of standardized morphology atlases in routine practice.

Taken together, the evaluation of laboratory performance in PBS examination provides clear evidence of significant variability across laboratories. While most achieved acceptable Z-scores for WBC count, differential counts, and platelet estimation, these results must be interpreted with caution. Highly scattered results inflated the standard deviation, broadening acceptable thresholds and potentially masking poor precision. In addition, the poor agreement between laboratory results and expert evaluations in identifying abnormal RBC morphologies and inclusions highlights inconsistencies in interpretive accuracy. These findings support the second research alternative hypothesis (H1,2), which proposed that there is significant variability in the performance of medical laboratories in the West Bank in PBS examination, and lead to the rejection of the corresponding null hypothesis (H0,2).

5.2 Summary

The findings of this study provide clear and consistent support for both proposed alternative hypotheses. First, the study revealed notable deficiencies in the knowledge of laboratory technicians, particularly in staining procedures, examination protocols, and morphological identification. These deficiencies were found to be influenced by technicians' background characteristics, including their roles in PBS testing, years of experience, and educational level. In addition, considerable variability in PBS-related practices was observed across laboratories, particularly in smear preparation, staining protocols, and microscopy usage. Although these practices were not analyzed statistically in relation to background characteristics, the descriptive findings highlight inconsistent adherence to standard guidelines.

These results support the first alternative hypothesis (H1,1) and justify the rejection of the first null hypothesis (H0,1), confirming that significant deficiencies in knowledge and variability in practices are present across laboratory settings.

Furthermore, the study confirmed significant variability in laboratory performance in PBS examination across the West Bank. Although most laboratories achieved acceptable Z-scores, inflated standard deviations broadened the range of acceptable results and may have masked poor analytical precision. Additionally, limited agreement with expert evaluations in identifying abnormal RBC morphology underscores gaps in interpretive accuracy. These findings support the second alternative hypothesis (H1,2) and lead to the rejection of the second null hypothesis (H0,2), which assumed no significant variability in performance.

5.3 Conclusions

This study provides a comprehensive evaluation of the current status of PBS testing in clinical laboratories across Palestine, with a focus on knowledge, practical implementation, and laboratory performance in PBS examination. The findings revealed low to moderate overall knowledge levels among laboratory technicians, with notable deficiencies in the staining process, examination techniques, and

identification of blood cell morphology. These knowledge gaps were found to be influenced by technician roles, years of experience, and educational background.

Substantial variability was also observed in PBS-related practices, including smear preparation, staining protocols, and examination methods. These inconsistencies point to a lack of standardized procedures and reflect limited access to structured training and external quality oversight.

The evaluation of laboratory performance further confirmed significant variability, particularly in WBC and platelet count estimations, as well as in the examination of abnormal RBC morphology and inclusions. Although many laboratories achieved acceptable Z-scores, the presence of inflated standard deviations and poor agreement with expert evaluations suggest weaknesses in both precision and interpretive accuracy.

Collectively, these findings support the study's hypotheses and reinforce the urgent need to establish a national EQAS specifically focused on PBS examination performance evaluation. The results also emphasize the importance of standardized protocols, targeted training programs, continuous professional development, and strengthened quality assurance practices. These measures are essential to ensure diagnostic reliability, enhance laboratory competency, and move toward diagnostic excellence in PBS testing across Palestine.

5.4 Limitations

This study is subject to several limitations. First, the knowledge and practice assessments were based on self-reported responses collected through a questionnaire, which may be affected by response bias or social desirability, potentially leading participants to report more favorable practices than those actually performed. Second, participation in the performance evaluation part was voluntary and limited to laboratories that agreed to be part of the study. This may have introduced selection bias, as laboratories with greater interest may have been more likely to participate. Third, the relatively small number of participants, particularly in the performance evaluation part, limits the results from being generalized to the wider laboratory

community. Fourth, the cross-sectional design of the study restricts the ability to establish causal relationships between technician knowledge, practice quality, and laboratory performance; instead, only associations can be concluded. Finally, the performance evaluation component focused on selected aspects of PBS testing using a limited number of pre-stained blood smears with mild abnormalities. As a result, the full scope of laboratory diagnostic capabilities—especially in detecting rare or more complicated morphological abnormalities—may not have been comprehensively assessed.

Despite these limitations, this study provides valuable baseline data and offers important understanding into current PBS examination practices in Palestine. It highlights critical areas for improvement and emphasizes the need for procedure standardization, training, and quality assurance programs to enhance diagnostic accuracy and consistency.

5.5 Recommendations

1. Establish a national EQAS program for PBS examination to ensure the reliability of laboratory results.
2. Develop and implement standardized protocols for PBS preparation, staining, and examination based on internationally recognized practices and guidelines.
3. Introduce periodic training programs for laboratory technicians, with a special focus on the staining process, counting approaches, morphological interpretation, and quality control procedures.
4. Conduct follow-up studies to assess the long-term impact of education, standardization, training and EQAS implementation on laboratory performance.

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Appendices

Appendix 3.1

English Research Survey

Survey Form to Determine the Sample of Participants in a Scientific Research Study

1. Governorate: _____

2. Laboratory Name: _____

3. Laboratory Manager: _____

4. Phone Number: _____

6. Does your lab accept requests for blood smear testing?

- Yes
- No

7. If yes, what is the nature of your work with the test?

- Preparing and reading the slides in the same laboratory.
- Preparing the slides in the laboratory and sending them to another entity for reading.
- Sending blood samples to another entity to prepare and read the slides.

Appendix 3.2

Arabic Research Survey

نموذج مسحي لتحديد عينة المشاركين في بحث علمي

١. المحافظة: _____

٢. اسم المختبر: _____

٣. مسؤول المختبر: _____

٤. رقم الهاتف: _____

٦. هل تقوم ب استقبال طلبات فحص شرائح الدم في مختبرك؟

- نعم
- لا

٧. إذا كان الجواب نعم، ما طبيعة عملك للفحص؟

- يتم تحضير الشرائح وقراءتها في نفس المختبر
- يتم تحضير الشرائح داخل المختبر، وارسال الشرائح الى جهة اخرى لقراءتها
- ارسال عينات الدم الى جهة اخرى ليتم تحضير منها شرائح وقراءتها

Appendix 3.3

PBS Examination Form

<table>
<tr><th colspan="2">Slide No.1</th></tr>
<tr><th colspan="2">“White Blood cells Evaluation”</th></tr>
<tr><td>▪ WBC’s count estimate ……. X10^3/ µL</td><td>Estimate the WBC’s count.</td></tr>
<tr><td>▪ WBC’s differential:
Neutrophils …….. %
Lymphocytes…….... %
Eosinophils ……... % Basophils ..……. %
Monocytes …..…... % Band Neutrophils……%
Hypersegmented Neutrophils……%
Immature forms……. %</td><td>Do white blood cells differential in 100 WBC’s.</td></tr>
<tr><th colspan="2">“Red Blood cells Evaluation”</th></tr>
<tr><td>▪ Nucleated RBC’s…… / 100 WBC</td><td>How many NRBC’s in 100 WBC’s</td></tr>
<tr><td>▪ Polychromasia (Reticulocytosis)…….. +</td><td>Use the following scale:
<table>
<tr><th>Slight</th><th>1+</th><th>2+</th><th>3+</th><th>4+</th></tr>
<tr><td>1 %</td><td>2 %</td><td>5 %</td><td>10 %</td><td>> 11%</td></tr>
</table></td></tr>
<tr><td>▪ Size:
Microcytes……..... +
Macrocytes……..… +</td><td>Use the following scale:
<table>
<tr><th>Normal</th><th>Slight</th><th>1+</th><th>2+</th><th>3+</th><th>4+</th></tr>
<tr><td>5%</td><td>(5-10) %</td><td>(10-25) %</td><td>(25-50) %</td><td>(50-75) %</td><td>> 75%</td></tr>
</table></td></tr>
<tr><td>▪ Hb content:
a. Normochromic……..... %
Hypochromic……..….. %

b. Degree of hypochromia……. +</td><td>use the following scale for degree of hypochromia:
<table>
<tr><td>1+</td><td>Area of central pallor is one-half of cell diameter</td></tr>
<tr><td>2+</td><td>Area of pallor is two-thirds of cell diameter</td></tr>
<tr><td>3+</td><td>Area of pallor is three-quarters</td></tr>
<tr><td>4+</td><td>Thin rim of hemoglobin</td></tr>
</table></td></tr>
</table>

<table>
<tr>
<td>

▪ **Shape:**

a. Abnormal forms **……. %**

b. Abnormal forms grading:

☐ pherocytes…….... + ☐

S☐ tocytes……+ ☐

Tear drop…… + Sickle

c☐ ……..+ ☐

Target cells……..+

☐ ☐ canthocytes

……..+

Bites cells……+

☐ Ovalocytes…

……..+

Stomatocytes…...…+ ☐

Echinocytes

……+

</td>
<td>

Use the following scale for Abnormal forms grading:

Normal	Slight	1+	2+	3+	4+
5%	(5-10) %	(10-25) %	(25-50) %	(50-75) %	> 75%

</td>
</tr>
<tr>
<td>

▪ **Inclusions:**

☐ owell-Jolly ☐ appenheimer

H☐ bodies ☐

Basophilic stippling Cabot ring

Hb crystals

</td>
<td>

Which red blood cells inclusions do you see?

☐

☐

</td>
</tr>
<tr>
<td colspan="2">“Platelets”</td>
</tr>
<tr>
<td>

▪ **Platelets count estimate…….. X10^3/ µL**

</td>
<td>

Estimate the Platelets count.

</td>
</tr>
<tr>
<td>

▪ **Platelets size and morphology:**

a. Size variation ……..…..

b. Shape variation…….

</td>
<td>

Use the following scale:

Normal	Slight	1+	2+	3+	4+
5%	(5-10) %	(10-25) %	(25-50) %	(50-75) %	> 75%

</td>
</tr>
</table>

Appendix 3.4

English Study Questionnaire

Assessment of Knowledge and Practices in Blood Smear Preparation, Staining, and Examination Among Laboratory Technicians in the West Bank

Section One: Demographic and Background Characteristics

This section contains the demographic data of the study participants. Please answer the following questions:

1. Governorate: _____
2. Type of Laboratory You Work In:
   - Medical Laboratory / Independent
   - Medical Laboratory / Hospital
   - Medical Laboratory / Primary Health Care Center
   - Research Laboratory
   - Specialized Laboratory. Specify: _____
3. Educational Qualification:
   - Diploma
   - Bachelor's Degree
   - Master's Degree
   - Doctorate
4. Years of Experience:
   - Less than 6
   - 6–10
   - 11–15
   - More than 15
5. Nature of Your Work in Blood Smear Testing:
   - I do not conduct this test
   - I only stain the slides and send them to another entity for reading
   -I prepare, stain, and read the slides in my laboratory

Section Two: Knowledge about Blood Smear Preparation, Staining, and Examination

This section aims to assess the knowledge of laboratory technicians in the preparation and staining of blood smears, as well as their ability to read stained slides and identify the shapes and types of different blood cells. Please answer the following questions based on your knowledge and experience without using any external aids or searching for model answers.

A: Knowledge about Sample handling and preparation

6. What type of anticoagulant is best for drawing blood samples for blood smear testing?
   - EDTA
   - Citrate
   - Heparin
   - Other: _____

7. What is the ideal time to start preparing blood slides after drawing the sample?
   - Immediately after drawing the sample
   - 2–4 hours
   - 4–6 hours
   - >6 hours

8. What is the technique used to make a blood smear suitable for analyzing the blood cells in terms of type, shape, and quantity?
   - Thin blood smear method
   - Thick blood smear method

9. After placing the blood on the glass slide, what is the optimal angle to spread the smear?
   - 25°
   - 45°
   - 65°
   - 85°

10. After spreading the sample on the glass slide:
   - Air dry, then add fixer
   - Add fixer immediately after spreading the blood on the slide
   - Add stain immediately after spreading the blood on the slide

B: Knowledge about PBS Staining Process

11. What type of stains are used to make blood smears?

- Prussian blue stain
- Papanicolaou stains
- Romanowsky stains
- New methylene blue stain
- Hematoxylin stain

12. What are the main ingredients in the stains used to make a blood slide? (You can choose more than one answer)

- Eosin
- Carbol fuchsin
- Methylene blue
- Safranin

13. What is the best fixative material(s) to use in preparing blood slides? (You can choose more than one option)

- 100% Ethanol
- 75% Isopropanol
- 90% Ethanol
- Absolute Methanol

14. After the staining time on the slide is complete:

- Excess stain is dried from the slide with drying paper
- The slide is washed with tap water and left to air dry
- The slide is washed with alcohol and left to air dry

C: Knowledge about Blood Smear Examination

15. What is the appropriate microscopic lens used to examine the general quality of the stained slide, such as the distribution of cells on the slide or the presence of any clumps of different blood cells?

- Low power objective lens 10x
- High power objective lens 40x
- Oil-immersion objective lens 100x

16. What is the appropriate microscopic lens used to determine the WBC estimate, correlate it with the WBC count from the CBC test, evaluate the morphology of the WBCs, and record any abnormalities, such as granulation?

- Low power objective lens 10x
- High power objective lens 40x
- Oil-immersion objective lens 100x

17. How many microscopic fields need to be read to evaluate the white blood cell estimate?
- 10
- 20
- 100
- 200

18. What is the appropriate microscopic lens used to make a WBC differential?
- Low power objective lens 10x
- High power objective lens 40x
- Oil-immersion objective lens 100x

19. How many white blood cells must be counted for a manual WBC differentiation test?
- 10
- 50
- 100
- 200

20. The white blood cell count should be corrected when the number of nucleated red blood cells is:
- >5 NRBCs
- >10 NRBCs
- >15 NRBCs
- >20 NRBCs

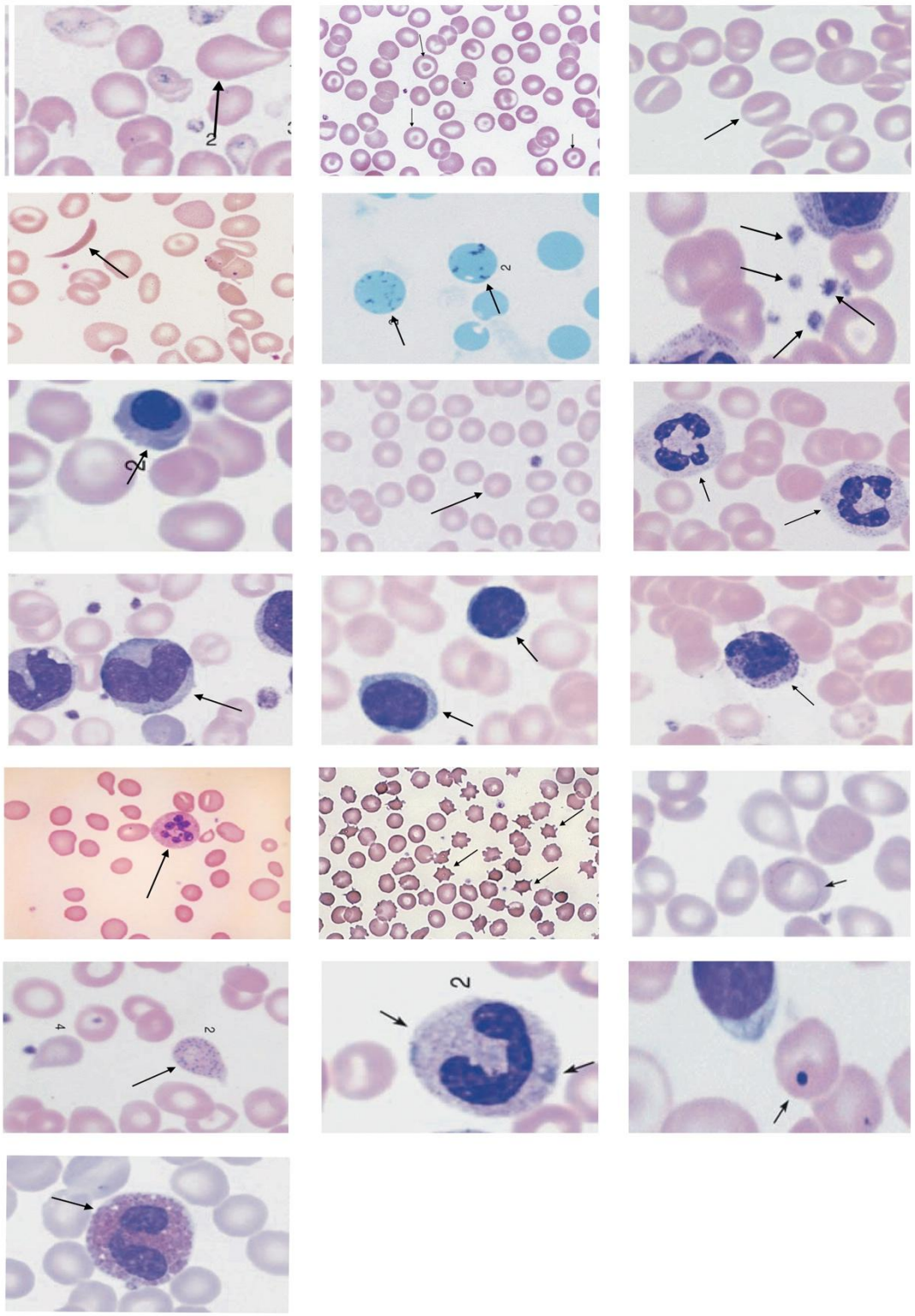
21. What is the appropriate microscopic lens used to evaluate the inclusions, shape, and size of RBCs?
- Low power objective lens 10x
- High power objective lens 40x
- Oil-immersion objective lens 100x

22. What is the appropriate microscopic lens used to perform an estimated platelet count and evaluate its morphology?
- Low power objective lens 10x
- High power objective lens 40x

- Oil-immersion objective lens 100x

D: Knowledge about Blood Cell Morphology

23. Identify the type of cell indicated by the arrow: (from 23.1 – 23.19)



Section Three: Procedures for Preparing and Staining Blood Slides in Your Laboratory

This section aims to highlight the nature of blood smear examination in your laboratory and the methods used for preparing and staining slides. Please answer the following questions, ensuring that your responses accurately reflect the procedures followed in your laboratory.

A. Nature of the PBS Examination Work in Your Laboratory

24. Have you received specialized training for preparing and staining blood slides?
  - Yes
  - No

25. How often do you prepare blood smears?
  - Daily
  - Weekly
  - Monthly
  - Annually

26. When do you perform or request a blood smear test? (You can choose more than one answer)
  - Only upon doctor's request
  - To confirm an abnormal CBC result
  - When there is a “flag” or questionable CBC result

27. Do you evaluate the quality of stained smears in your laboratory?
  - Yes
  - No

28. Are you participating in an external quality control program for examining blood smears?
  - No
  - Yes, please specify: _____

29. If you do not read the slides immediately after preparing them, do you apply a preservative on the slides?
  - Yes

- No

30. What is the time required to transfer a stained blood slide to another facility for reading?

- Within 2 hours
- 2 – 4 hours
- 4 – 6 hours
- More than 6 hours
- Slides are read in the same laboratory

31. The method you use to transport stained slides:

- Special box with ice
- Special box without ice
- Without a special box
- I do not transport slides outside the laboratory

B. Preparation and Staining Blood Slides in Your Laboratory

32. The method used to stain blood smears in your laboratory:

- Manual method
- Automated method

33. The type of anticoagulant used to draw blood samples for blood smear testing in your laboratory:

- EDTA
- Citrate
- Heparin
- Other: _____

34. If the type of anticoagulant used is EDTA, what is its chemical composition?

- K2-EDTA
- K3-EDTA
- Na2-EDTA
- Other: _____

35. When do you start preparing slides after collecting the sample?

- Within 2 hours
- 2 – 4 hours
- 4 – 6 hours

- More than 6 hours

36. After placing the blood on the slide, how long do you leave the slide to dry?
- Within 2 hours
- 2 – 4 hours
- 4 – 6 hours
- More than 6 hours

37. What type of fixative agent do you use?
- 90% ethanol
- 100% ethanol
- 100% methanol
- Other: _____

38. Duration of fixation (in minutes):
- _____ minutes

39. After the fixation process is complete, how long do you wait before placing the stain on the slide?
- Less than 6 hours
- 6 – 12 hours
- 12 – 18 hours
- More than 18 hours

40. The type of stain used in your laboratory:
- Wright stain
- Giemsa stain
- Leishman stain
- Diff-Quick
- Other: _____

41. Duration of staining (in minutes):
- _____ minutes

Section Four: Blood Smear Examination in Your Laboratory

This section aims to understand the process of blood smear examination in your laboratory. Please answer the following questions, ensuring that your responses accurately reflect the practices followed in your laboratory.

A. Reading Blood Smears

42. Which microscopic lens do you use to determine the WBC estimate, correlate it with the WBC counts from automated instruments, evaluate the morphology of the WBCs, and record any abnormalities, such as granulation?

- Low power objective lens 10x
- High power objective lens 40x
- Oil-immersion objective lens 100x

43. How many microscopic fields need to be read to evaluate the white blood cell estimate?

- _____ field(s)

44. Which microscopic lens do you use to make a WBC differential?

- Low power objective lens 10x
- High power objective lens 40x
- Oil-immersion objective lens 100x

45. How many white blood cells do you count for a manual white blood cell differential?

- _____ WBCs

46. You correct the WBC count when the number of nucleated red blood cells is more than:

- NRBCs > _____

47. Which microscopic lens do you use to evaluate the inclusions, shape, and size of RBCs?

- Low power objective lens 10x
- High power objective lens 40x
- Oil-immersion objective lens 100x

48. Which microscopic lens do you use to perform an estimated platelet count and evaluate its morphology?

- Low power objective lens 10x
- High power objective lens 40x
- Oil-immersion objective lens 100x

Appendix 3.5

Arabic Study Questionnaire

القسم الأول: البيانات الديموغرافية

يحتوي هذا القسم على البينات الديموغرافية الخاصة بالمشاركين في الدراسة. الرجاء الإجابة على الأسئلة التالية:

.١ المحافظة _____

.٢ نوع المختبر الذي تعمل فيه

- مختبر طبي / مستقل
- مختبر طبي / مستشفى
- مختبر طبي / مركز رعاية صحية أولية
- مختبر بحثي
- مختبر متخصص. حدد _____

.٣ المؤهل العلمي

- دبلوم
- درجة بكاليوس
- درجة ماجستير
- دكتوراه

.٤ سنوات الخبرة

- 6 أقل من
- 6–10
- 11–15
- 15 أكثر من

.٥ طبيعة عملك لفحص شريحة الدم

- لا أجرى هذا الاختبار
- أكتفي بتلوين الشرائح وإرسالها إلى جهة أخرى للقراءة
- أعد وألصق وأقرأ الشرائح في مختبري

القسم الثاني: المدى المعرفي

يستهدف هذا القسم تقييم مدى معرفة فنيي المختبرات بآلية تحضير وصباغة شرائح الدم، والمقدرة على قراءة الشرائح المصبوغة والتعرف على اشكال وأنواع خلايا الدم المختلفة. الرجاء الإجابة على الأسئلة التالية بحسب ما تمتلك من معلومات وخبرات، وعدم الاستعانة بوسائل مساعدة او البحث عن الإجابة النموذجية.

أ. تقييم المدى المعرفي لعملية تحضير العينة المناسبة لفحص شريحة الدم

.؟ ٦ما هو نوع مانع التخثر الأفضل لسحب عينات الدم لاختبار شريحة الدم

EDTA-

- سترات

- الهيبارين

- _____ أخرى

.؟ ٧ما هو الوقت المثالي لبدء إعداد الشرائح بعد سحب العينة

- مباشرة بعد سحب العينة

- ساعات 4–2

- ساعات 6–4

– ساعات 6<

.؟ ٨ما هي التقنية المستخدمة لجعل شريحة الدم مناسبة لتحليل خلايا الدم من حيث النوع والشكل والكمية

Thin blood smear method -

Thick blood smear method -

.؟ ٩بعد وضع الدم على الشريحة الزجاجية، ما هو الزاوية المثالية لتمديد الشريحة

25° -

45° -

65° -

85° -

١٠. بعد سحب مسحة الدم على الشريحة الزجاجية

- تترك لتجف في الهواء، ثم يضاف المثبت

- يضاف المثبت فوراً بعد تمديد الدم على الشريحة

- يضاف الصبغة فوراً بعد تمديد الدم على الشريحة

ب. تقييم المدى المعرفي لعملية صباغة الشرائح

.؟ ١١ما نوع الصبغات المستخدمة لعمل شريحة الدم

- Prussian blue stain

- Papanicolaou stains

- Romanowsky stains

- New methylene blue stain

- Hematoxylin stain

.١٢)ما هي المكونات الرئيسية في الصبغات المستخدمة لصنع شريحة دم؟ (يمكنك اختيار أكثر من إجابة

- Eosin

- Carbol fuchsin

- Methylene blue

- Safranin

.١٣)ما هو أفضل مادة مثبتة تستخدم في إعداد شرائح الدم؟ (يمكنك اختيار أكثر من خيار

- إيثانول 100%

- إيزوبروبانول 75%

- إيثانول 90%

- 100% ميثانول

.١٤ بعد انتهاء وقت التلوين على الشريحة

- يتم تجفيف الصبغة الزائدة من الشريحة باستخدام ورق تجفيف

- يتم غسل الشريحة بالماء العادي وتركها لتجف في الهواء

- يتم غسل الشريحة بالكحول وتركها لتجف في الهواء

١٥. ما هي عدسة المجهر المناسبة المستخدمة لفحص الجودة العامة للشريحة المصبوغة مثل توزيع الخلايا على الشريحة او وجود أي تكتلات لخلايا الدم المختلفة؟

١٠ العدسة الشيئية منخفضة القوة -

٤٠ العدسة الشيئية مرتفعة القوة -

١٠٠ العدسة الشيئية الزيتية -

ج. تقييم المدى المعرفي لعملية قراءة شريحة الدم

١٦. ما هي عدسة المجهر المناسبة المستخدمة لتقييم خلايا الدم البيضاء من حيث العدد، تأكيد نتيجة عدد خلايا الدم البيضاء من فحص CBC، الشكل والمكونات الداخلية الغير طبيعية للخلايا البيضاء؟

١٠ العدسة الشيئية منخفضة القوة -

٤٠ العدسة الشيئية مرتفعة القوة -

١٠٠ العدسة الشيئية الزيتية -

١٧. كم عدد الحقول المجهرية التي يجيب قراءتها لتقييم عدد خلايا الدم البيضاء؟

10 -

20 -

100 -

200 –

١٨. ما هي عدسة المجهر المناسبة المستخدمة لعمل تمايز لخلايا الدم البيضاء؟

١٠ العدسة الشيئية منخفضة القوة -

٤٠ العدسة الشيئية مرتفعة القوة -

١٠٠ العدسة الشيئية الزيتية -

١٩. كم خلية دم بيضاء يجب عدها لعمل فحص تمايز خلايا الدم البيضاء؟

10 -

50 -

100 -

200 -

٢٠. يجب تصحيح عدد خلايا الدم البيضاء عندما يكون عدد خلايا الدم الحمراء ذات الانوية:

>5 NRBCs -

>10 NRBCs -

>15 NRBCs -

>20 NRBCs –

٢١. ما هي عدسة المجهر المناسبة المستخدمة لتقييم خلايا الدم الحمراء من حيث الشكل والحجم والمكونات؟

١٠ العدسة الشيئية منخفضة القوة -

٤٠ العدسة الشيئية مرتفعة القوة -

١٠٠ العدسة الشيئية الزيتية -

٢٢. ما هي عدسة المجهر المستخدمة لتقييم شكل وعدد صفائح الدم؟

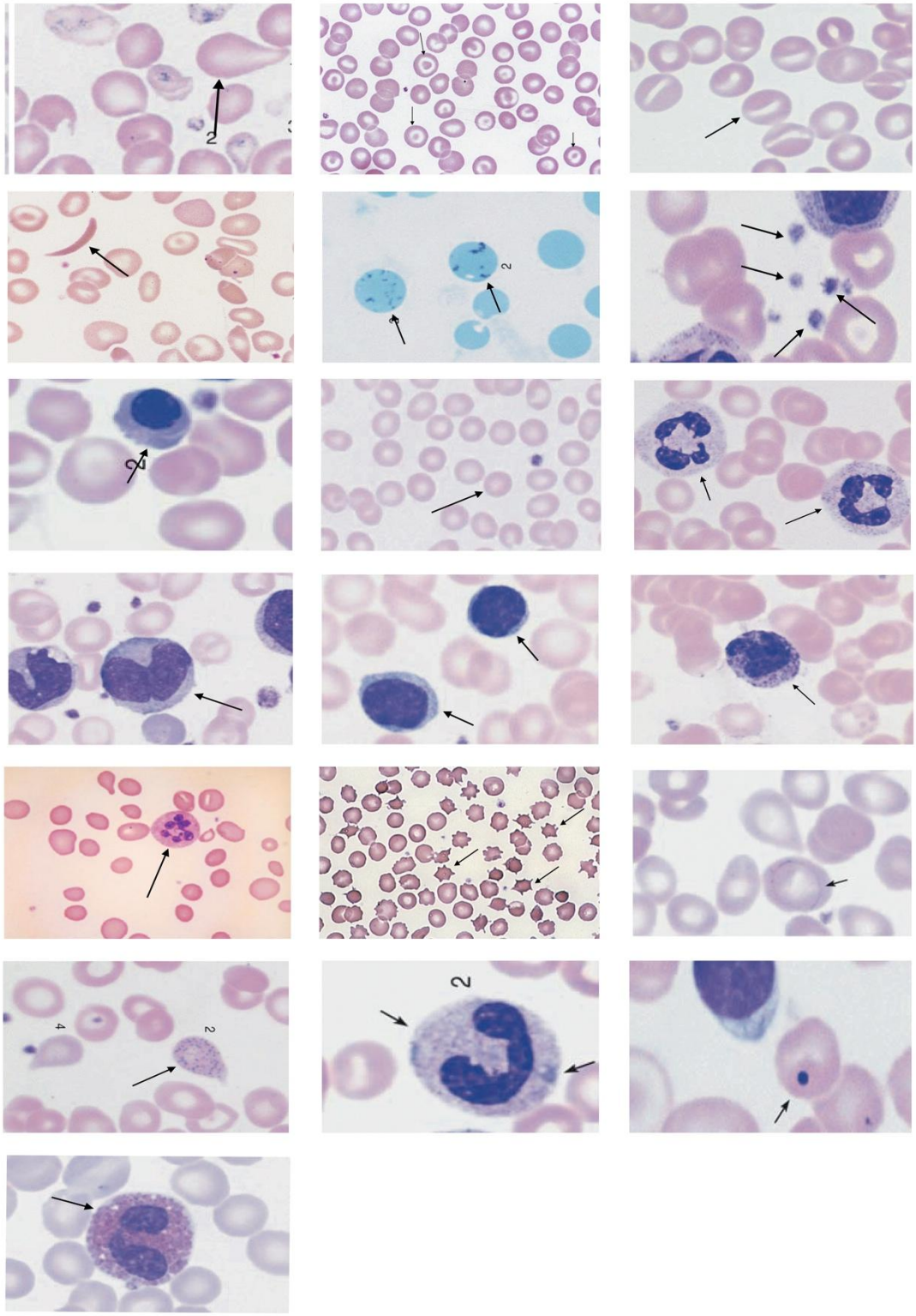
١٠ العدسة الشيئية منخفضة القوة -

٤٠ العدسة الشيئية مرتفعة القوة -

١٠٠ العدسة الشيئية الزيتية -

د. تقييم المدى المعرفي بأشكال خلايا الدم المختلفة

٢٣) ٢٣ حدد نوع الخلية المشار إليه بالسهم (من ١.٢٣ الى ١٩.٢٣.



القسم الثالث: إجراءات تحضير وصباغة شرائح الدم في مختبركم.

يهدف هذا القسم إلى تسليط الضوء على طبيعة عمل الفحص في مختبركم، والآلية المتبعة لتحضير وصباغة الشرائح، الرجاء الإجابة على الأسئلة التالية مع مراعاة أن تكون الإجابات تعكس تماما طريقة العمل المتبعة في مختبركم.

أ. طبيعة عمل الفحص في مختبركم.

.؟ ٢٤هل تلقيت تدريبًا متخصصًا لإعداد وتلوين شرائح الدم

- نعم

- لا

؟ما هو تكرار المرات التي تقوم بها بتحضير شريحة دم .٢٥

- يوميًا

- أسبوعيًا

- شهريًا

- سنويًا

.٢٦)متى تقوم بإجراء أو طلب اختبار مسحة دم؟ (يمكنك اختيار أكثر من إجابة

فقط بناءا على طلب الطبيب -

لتأكيد نتيجة فحص الدم الكامل -

– عندما تظهر إشارة تحذيرية في فحص الدم الكامل

٢٧. هل تقوم بعمل تقييم لجودة صباغة شرائح الدم في مختبرك؟

- نعم

- لا

.٢٨هل انت مشترك في برنامج رقابة نوعية خارجي خاص بفحص شرائح الدم؟

- لا

- نعم، يرجى التحديد _____

٢٩. في حال عدم قراءة الشرائح فور تحضيرها، هل تقوم بوضع مادة حافظة على الشرائح؟

- نعم

- لا

٣٠. ما المدة الزمنية التي تحتاجها لنقل شريحة الدم المصبوغة الى جهة أخرى ليتم قراءتها؟

- خلال ساعتين

- ساعات 4 – 2

- ساعات 6 – 4

- أكثر من 6 ساعات

- تتم قراءة الشرائح في نفس المختبر

٣١. الطريقة التي تقوم بها بنقل الشرائح المصبوغة:

- صندوق خاص مع ثلج

- صندوق خاص بدون ثلج

- بدون صندوق خاص

- لا أنقل الشرائح خارج المختبر

ب. البيانات التقنية الخاصة بطريقة تحضير وصباغة شرائح الدم في مختبركم.

٣٢. الطريقة المستخدمة في صباغة شرائح الدم في مختبركم:

- طريقة يدوية
- طريقة آلية

٣٣. نوع مانع التجلط المستخدم لسحب عينات الدم لعمل فحص شرائح الدم في مختبركم:

- EDTA
- سترات
- الهيبارين
- أخرى _____

٣٤. إذا كان نوع مانع التجلط المستخدم EDTA، ما هي التركيبة الكيميائية له؟

- K2EDTA
- K3EDTA
- Na2EDTA
- أخرى _____

٣٥. متى تقوم ب البدء بتحضير الشرائح بعد سحب العينة؟

- خلال ساعتين
- ساعات 4 – 2
- ساعات 6 – 4
- أكثر من 6 ساعات

٣٦. بعد وضع عينة الدم على الشريحة الزجاجية، كم من الوقت تترك الشريحة لتجف؟

- خلال ساعتين
- ساعات 4 – 2
- ساعات 6 – 4
- أكثر من 6 ساعات

٣٧. ما نوع المادة المثبتة التي تستخدمها؟

- إيثانول 90%
- إيثانول 100%
- ميثانول 100%
- _____ أخرى

٣٨. مدة وضع المثبت على الشريحة (بالدقائق):

دقائق _____

٣٩.بعد الانتهاء من مرحلة تثبيت العينة بالمادة المثبتة، كم تستغرق من الوقت لوضع الصبغة على الشريحة:

- أقل من 6 ساعات

- ساعة 12 – 6
- ساعة 18 – 12
- أكثر من 18 ساعة

٤٠. نوع الصبغة المستخدمة في مختبركم:
- صبغة رايت
- صبغة غيمسا
- صبغة ليشمان
- ديف كويك
- _____ أخرى

٤١. مدة وضع الصبغة على الشريحة (بالدقائق):
دقائق _____

القسم الرابع: قراءة شرائح الدم المصبوغة في مختبركم.
يهدف هذا القسم الى التعرف على آلية قراءة شرائح الدم المصبوغة في مختبركم، الرجاء الإجابة على الأسئلة التالية مع مراعاة ان تكون الإجابات تعكس تماما ما يتم العمل به في مختبركم.
أ. قراءة شريحة الدم.
٤٢. ما هي عدسة المجهر التي تستخدمها لتقييم خلايا الدم البيضاء من حيث العدد، تأكيد نتيجة عدد خلايا الدم البيضاء من فحص CBC، الشكل والمكونات الداخلية الغير طبيعية للخلايا البيضاء؟
- ١٠ العدسة الشيئية منخفضة القوة
- ٤٠ العدسة الشيئية مرتفعة القوة
- ١٠٠ العدسة الشيئية الزيتية

٤٣. كم عدد الحقول المجهرية التي تقرءها لتقييم عدد خلايا الدم البيضاء؟
_____ حقل

٤٤. ما هي عدسة المجهر التي تستخدمها لعمل تمايز لخلايا الدم البيضاء؟
- ١٠ العدسة الشيئية منخفضة القوة
- ٤٠ العدسة الشيئية مرتفعة القوة
- ١٠٠ العدسة الشيئية الزيتية

٤٥. كم خلية دم بيضاء تقوم ب عدها لعمل فحص تمايز خلايا الدم البيضاء؟
كريات دم بيضاء _____

٤٦. تقوم بتصحيح عدد خلايا الدم البيضاء عندما يكون عدد خلايا الدم الحمراء ذات الانوية اكثر من:
NRBCs > _____

٤٧. ما هي عدسة المجهر التي تستخدمها لتقييم خلايا الدم الحمراء من حيث الشكل والحجم والمكونات؟
- ١٠ العدسة الشيئية منخفضة القوة

٤٠ العدسة الشيئية مرتفعة القوة -

١٠٠ العدسة الشيئية الزيتية -

٤٨. ما هي عدسة المجهر التي تستخدمها لتقييم شكل وعدد صفائح الدم؟

١٠ العدسة الشيئية منخفضة القوة -

٤٠ العدسة الشيئية مرتفعة القوة -

١٠٠ العدسة الشيئية الزيتية -

Appendix 3.6

IRB Approval

Al Quds University
Faculty of Health Professions
Jerusalem –Abu Dis

جامعة القدس
كلية المهن الصحية
القدس – أبو ديس

Research Ethics Subcommittee of Faculty of Health Professions
Letter of approval

April 9, 2025
Ref. No.: RESC/2025-62

Dear Applicants, (**Prof. Rania Abu Seir, Mr. Wisam Alshalash**)

Program: **MSc Medical Labs Department**

The Research Ethics subcommittee of the Faculty of Health Professions has recently reviewed your proposal entitled (**Quality Assessment of Peripheral Blood Smear: A Comprehensive Analysis of Examination and Preparation Quality in Clinical Laboratories in Palestine**) submitted by (**Prof. Rania Abu Seir**). Your proposal is deemed to meet the requirements of research ethics at Al-Quds University, but further assessment is required by the Central Research Ethics Committee of Al-Quds University. We wish you all best for the conduct of the project.

Hussein ALMasri, PhD
Associate Professor of Medical Imaging
Research Ethics Subcommittee Chair
Faculty of Health Professions

Hussein ALMasri

CC: File
CC: Committee members

Tel. Fax: 02 2791243 **Email: dean@hpro.alquds.edu** **تلفاكس: 02 2791243**

Appendix 3.7

MOH Approval

State of Palestine
Ministry of Health
Assistant Deputy for Allied Medical
Professions and Blood Banks

دولة فلسطين
وزارة الصحـــــــة
الوكيل المساعد
للمهن الطبية المساندة وبنوك الدم

عطوفة الأخ الدكتور كمال الشخرة المحتـــــــــــرم
الوكيل المساعد لشؤون الصحة العامة وصحة الاسرة
الاخ الدكتور معتصم محيسن المحتـــــــــــرم
الوكيل المساعد لشؤون المستشفيات والطوارئ

الموضوع:تسهيل مهمة

تحية طيبة وبعد,,,

بعـد التحيـة وبالاشـارة للموضـوع أعـلاه، يرجـى تسـهيل مهمـة الباحثـة الـدكتورة رانيـة ابـو سير من جامعة القدس

بعنوان: Quality Assessment of Peripheral Blood Smear:A comprehensive analysis of examination and preparation quality in clinical laboratories in Palestine

والتي تهدف الى تقديم تقييم شامل عن جودة قراءة فحص مسحة الدم في المختبرات الطبية في الضفة الغربية حيث سيتم العمل على تحديد نقاط الضعف في هذا الفحص والعمل على تحسينها وسيتم ذلك من خلال عمل مسح ميداني لمختبرات الضفة الغربية لتحديد المختبرات التي تقوم بعمل هذا الفحص حيث سيتم تحضير شرائح مسحة الدم من قبل مركز الجودة للمختبرات الطبية وستتم قراءة نتائج الفحص بواسطة مختصين في علم الدم (على اقل اثنين)ثم سيتم توزيع الشرائح على كل مختبر لتتم قراءتها بواسطة الفني المسؤول وتقييم نتائج المختبرات بناء على المقارنة بين نتائج قراءة المختصين.

على ان يتم التعامل مع كافة المعلومات بسرية تامة وتستخدم لأغراض البحث العلمي فقط.

وتفضلوا بقبول فائق الاحترام,,,

أخوكم
أسامة النجار
الوكيـل المساعد
للمهن الطبية المساندة وبنوك الدم

صادر
دولة فلسطين
وزارة الصحة
مكتب الوكيل المساعد للمهن الطبية المساندة وبنوك الدم
صادر رقم: 2024-529
التاريخ: 13/5/2024

Lana

Ministry of Health – Ramallah
Ministry of Health - Nablus

TelFax: 022964402: تلفاكس
e-mail :parmed@moh.ps
TelFax: 09-2335821: تلفاكس

وزارة الصحة –رام الله
وزارة الصحة – نابلس

تقييم فحص شريحة الدم في فلسطين: خطوة نحو ضمان الجودة ودقة التشخيص

إعداد: وسام عبدالله احمد الشلش

إشراف: أ.د. رانية أبو سير

ملخص:

خلفية الدراسة :يُعد اختبار شريحة الدم أداة تشخيصية مهمة في علم أمراض الدم للكشف عن العديد من اضطرابات الدم والأورام الخبيثة. وفي فلسطين، يشير غياب برنامج رقابة نوعية خارجية خاص بفحص شرائح الدم إلى وجود فجوة كبيرة في ضمان جودة الفحوصات وتوحيد معاييرها. هدفت هذه الدراسة إلى تقييم المدى المعرفي والتقني لفنيي المختبرات الطبية فيما يتعلق بتحضير شرائح الدم وصبغها وقراءتها، بالإضافة إلى تقييم أداء المختبرات في عملية قراءة شرائح الدم المصبوغة، والمساهمة في إنشاء برنامج رقابة نوعية خارجية خاص بعملية قراءة شرائح الدم.

منهجية البحث: تم استخدام منهجية بحثية مقطعية متعددة الطرق، تضمنت جزأين: (١) تقييم المدى المعرفي والتقني لفنيي المختبرات الطبية فيما يتعلق باختبار شرائح الدم من خلال استبيان استهدف الفنيين المرخصين في مختلف أنحاء فلسطين، وشارك فيها ٢٣٥ فنيًا، و(٢) تقييم أداء المختبرات في قراءة شرائح الدم باستخدام ثلاث شرائح دم مصبوغة تم توزيعها على ٣٩ مختبرًا يقومون بتحضير وقراءة شرائح الدم، وتم تحديد هذه المختبرات من خلال مسح استقصائي مسبق. تم تقييم أداء المشاركين استنادًا إلى قيم Z لقياس التوافق بين نتائج المختبرات، ومعامل التفاوت (%CV) لقياس دقة تكرارية النتائج لدى المختبر الواحد وبين المختبرات.

النتائج: كشفت نتائج تقييم المدى المعرفي عن مستوى معرفي عالٍ في تحضير شرائح الدم (٩٢.٥٪ ± ١١.٣)، لكن تم تسجيل نقص واضح في المعرفة في عملية الصباغة (٥٦.٧٪ ± ٢١.٣)، وأساليب الفحص (٦٠.٧٪ ± ١٨.٤)، والتعرف على خلايا الدم (٦٤.١٪ ± ٢١.٠)، مع متوسط عام للمدى المعرفي بلغ (٦٥.٩٪ ± ١٤.٤). كما وُجد ارتباط بين ارتفاع المؤهلات الأكاديمية والمشاركة الفعلية في اختبار PBS وبين تحسن نتائج المدى المعرفي. كما تم رصد تباينات عملية في تقنيات الصباغة، التثبيت، التجفيف، واستخدام المجهر. أظهر تقييم الأداء حصول غالبية المختبرات على قيم Z مقبولة في معظم المعايير (٩٤.٣٪ في تعداد كريات الدم البيضاء، ٩٧.١٪ في العد التفريقي للخلايا اللمفاوية، و١٠٠٪ في تعداد الصفائح الدموية)، إلا أن تحليل معامل التباين (%CV) كشف عن تفاوت كبير داخل المختبرات وبينها. اما بخصوص تقييم اشكال الخلايا، فكان توافق نتائج المشتركين مع تقييم الخبراء منخفضًا بشكل ملحوظ، خاصة في تحديد الخلايا صغيرة الحجم (١١.٤٪)، والخلايا الكروية (٨.٦٪)، والخلايا البيضوية (١١.٤٪).

الاستنتاج والتوصيات: كشفت النتائج عن وجود قصور في المدى المعرفي لدي فنيي المختبرات حول فحص شريحة الدم، ووجد أيضا تفاوت ملحوظ في طريقة تحضير الشرائح وقراءتها. هذه النتائج تسلط الضوء على الحاجة الملحة لإنشاء برنامج رقابة نوعية خارجي خاص بفحص شرائح الدم، وذلك لضبط جودة عمل الفحص في المختبرات الطبية في فلسطين.

الكلمات المفتاحية: شرائح الدم، أداء المختبرات، تقييم الجودة، برنامج رقابة نوعية خارجية، أمراض الدم، فلسطين، المدى المعرفي، المدى التقني.