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Manual of
Hematology



Editor
Sanjeev Kumar Sharma

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1 Peripheral Smear

Jyoti Bajaj Sawhney

Q1. How should blood samples be collected?

Ans: Blood is drawn from a vein (venipuncture), usually from the inside of the elbow or the back of the hand. The phlebotomist should have hand-hygiene materials (soap and water or alcohol rub), well-fitting nonsterile gloves, single-use disposable needles, and syringes or lancing devices in sufficient numbers to ensure that each patient has a sterile needle and syringe or equivalent for each blood sampling. Clean the site with a 70% alcohol swab for 30 seconds and allow to dry completely (30 seconds). The vein should be entered swiftly at a 30° angle or less.[1]

Q2. Which anticoagulants are used in tube used for collecting blood samples?

Ans: Whole blood is collected in blood collection tubes or syringes that contain the appropriate anticoagulant to inhibit coagulation.

Routine hematology tests require a blood collection tube containing ethylenediaminetetraacetic acid (EDTA), a chelating anticoagulant. EDTA acts as a chelating agent to bind cofactor divalent cations (mainly calcium) to inhibit enzyme reactions involved in the clotting cascade specifically the conversion of prothrombin to thrombin and subsequent inhibition of the thrombolytic action on fibrinogen to fibrin necessary for clot formation. EDTA preserves the morphology of the cellular elements of blood, making it a satisfactory anticoagulant for hematologic studies. Blood collection tubes containing heparin, which stabilizes the red blood cell (RBC) membranes, are used for specialized hematology studies, such as red cell fragility tests and several specialized chemistry tests. Blood collection tubes containing sodium citrate are used for coagulation studies.[2]

Q3. What preanalytic variables should be monitored when evaluating a blood sample?

Ans: Laboratory results are dependent on the quality of the specimen analyzed. Preanalytical steps, the major source of mistakes in laboratory diagnostics, arise during patient preparation, sample collection, sample transportation, sample preparation, and sample storage. The preanalytical phase accounts for the majority of laboratory errors. It has been estimated that preanalytical errors account for more than two-thirds of all laboratory errors, while errors in the analytical phase and postanalytical phase account for one-third of all laboratory errors.[3]

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The most commonly reported types of preanalytical error are:

- Missing sample and/or test request
- Wrong or missing identification
- Contamination from infusion route
- Inappropriate containers
- Hemolyzed or clotted sample
- Number of samples with insufficient volumes
- Inappropriate blood to anticoagulant ratio
- Inappropriate transport and storage conditions

Q4. What information can be obtained from a blood smear?

Ans: The blood film is one of the world's most widely and frequently used tests.

Blood smear examination serves three important objectives:[4]

1. It serves as a quality control tool in verifying the results generated by the automated analyzers.
2. It allows for identification of abnormal/immature/atypical cells, if present.
3. It allows for recognition of clinically significant morphologic abnormalities, which the analyzers are incapable of either flagging or detecting and identifying.

Currently available automated hematology analyzers do not generate any reportable information about the presence of many of the:

- Red cell abnormalities such as elliptocytes/ovalocytes, target cells, sickle cells, acanthocytes, echinocytes, SC crystalloids, stomatocytes, tear drop cells, rouleaux, Howell–Jolly bodies, pappenheimer bodies, basophilic stippling, and intraerythrocytic organisms
- White cell abnormalities (Auer rods, toxic granulation, toxic vacuolization, Dohle bodies, hypogranular/agranular granulocytes intraleukocytic organisms, etc.)
- Platelet abnormalities (platelet satellitosis, abnormal granulation, and hypogranulation/agranulation bizarre platelets).

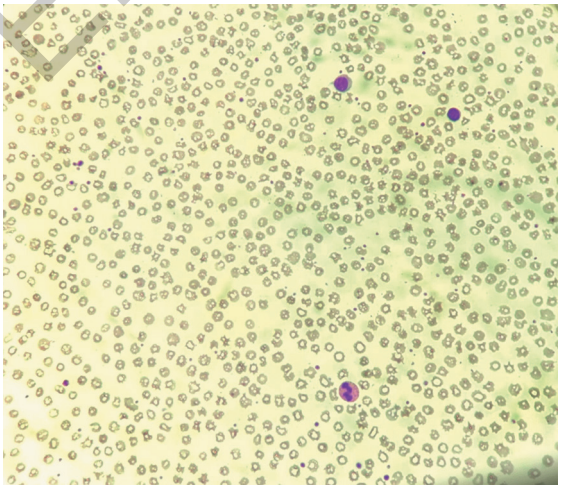

FIG. 1: Peripheral smear showing neutrophil and lymphocyte in the background of red blood cells (10×).

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Q5. What are the artifacts that can affect peripheral blood smears?

Ans: An artifact on peripheral blood smear refers to "an artificial structure or tissue alteration on a prepared microscopic slide as a result of an extraneous factor". They are the major source of diagnostic confusion. The most common artifacts on the peripheral smear are caused by poor spreading techniques, slow drying in humid conditions, insufficient or delayed fixation, and fixing solutions that contain water.

Certain cell types can be damaged or ruptured easily by the blood film preparation itself. These are called smudge cells.[5] These are associated with special conditions like those with large numbers of atypical lymphocytes, chronic lymphocytic leukemia, and acute leukemia.

Slow drying causes cells to contract, whereas, water in excess of 3% in the methanol fixing solution can cause the development of artificial vacuoles. Degenerative changes, such as cytoplasmic vacuolization in monocytes and neutrophils, nuclear lobulation, or fragmentation of nucleated cells, and apoptotic changes are caused by prolonged or inadequate sample storage.

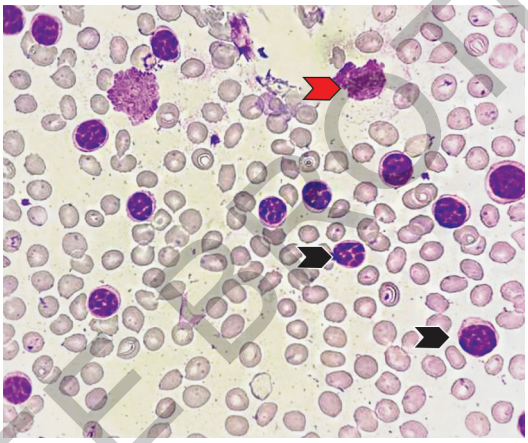

FIG. 2: Case of chronic lymphocytic leukemia: Peripheral smear (100×). Arrowhead (black)—mature lymphocytes with clumped chromatin resembling soccer ball. Arrowhead (red)—smudge cell.

Courtesy: Dr Abhishek Purohit.

Q6. What can be the false-positive and false-negative results from automated analyzers?

Ans:

- *White blood cells (WBCs)*: Causes of spurious decrease:[6]
  - Agglutination of polymorphonuclear leukocytes (PMN) (EDTA-related)
  - Agglutination of WBC other than PMN (lymphocytes, lymphoma cells, and leukemic blasts)
  - Excess amount of K3-EDTA anticoagulant
  - Coagulation within the sample
- *WBC*: Spurious increase—
  - Platelet aggregates
  - Very large platelet
  - Nucleated RBC
  - RBC resistant to lysis (newborns, abnormal Hb, chemotherapy, uremia, liver disease)

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  - Cryoglobulin, cryofibrinogen, and immunoglobulins
  - Lipids
  - Microorganisms (bacterial aggregates)
- *RBC*: Spurious decrease—
  - Cold agglutinins, warm agglutinins
  - Very small RBC
  - Cryoglobulins (↓ flow, inadequate aspiration)
  - In vitro hemolysis
  - Coagulation
- *RBC*: Spurious increase—
  - High WBC counts
  - Giant platelets
- *Hemoglobin: Spurious increase*—
  - Lipids
  - High WBC counts
  - Immunoglobulins (cryoglobulins)
  - In vitro hemolysis
  - Carboxyhemoglobin (high amount)
  - Bilirubin (>250–300 mg/L)
- *Hemoglobin*: Spurious decrease—
  - Coagulation within the sample
  - Overfilling vacuum tube
  - Venipuncture near a drip
  - Sulfhemoglobin
- *Mean corpuscular volume (MCV)*:
  - Cold agglutinins, warm agglutinins:↑
  - High WBC counts:↑
  - Hyperglycemia:↑
  - K2 EDTA in excess:↑
  - Hyper- or hyponatremia: ↑ or ↓

Conditions leading to possible interference with automated methods for reticulocyte analysis:

- *Inaccurate gating of RBCs*: Giant platelets, platelets clumps, abnormal WBCs, abnormal number of WBCs, WBC fragments, and nucleated RBCs
- *Intraerythrocytic particles*: Howell–Jolly bodies, pappenheimer bodies, basophilic stippling, Heinz bodies, sickle cells, spherocytes, hemoglobin H inclusions, *Plasmodium*, and *Babesia*
- *Others*: Cold agglutinin disease, autofluorescence of RBCs (drugs, porphyria), paraproteins, hemolysis, and diagnostic intravenous fluorescent dyes

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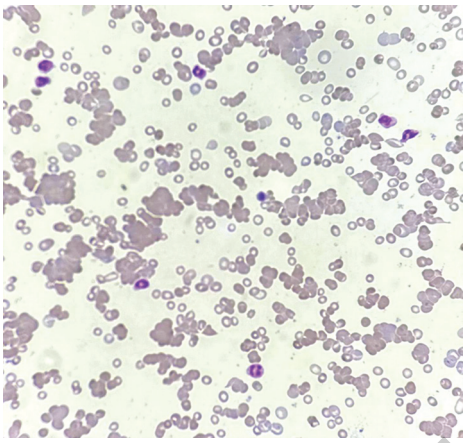

FIG. 3: Peripheral blood smear showing RBC agglutination.

Courtesy: Dr Abhishek Purohit.

Q7. What is rouleaux? What is its significance?

Ans: The RBCs in the plasma tend to form aggregates or rouleaux that appear similar to a stack of coins. Rouleaux formation is determined largely by increased levels of plasma fibrinogen and globulins. It is a reversible aggregation process. Although myeloma and macroglobulinemias are first considered by hematologists, other causes that occur more frequently include acute and chronic infections, connective tissue diseases, and chronic liver disease.[7]

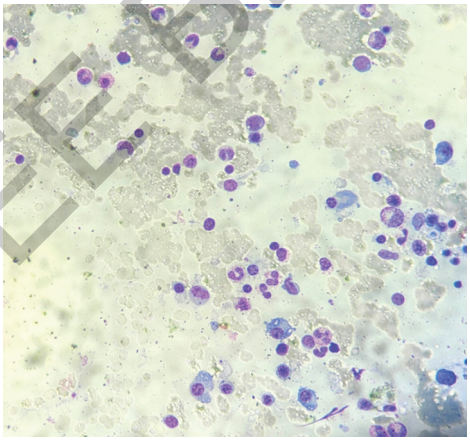

FIG. 4: Rouleaux formation in bone marrow aspirate in case of multiple myeloma with plasma cells.

Courtesy: Dr Jyoti Sawhney.

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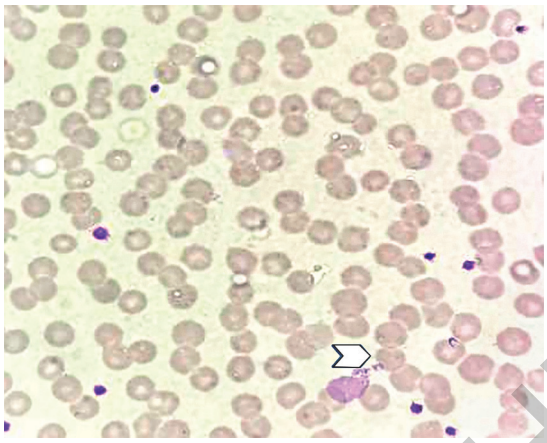

FIG. 5: Peripheral smear (100×): Rouleaux formation (arrowhead).

Courtesy: Dr Abhishek Purohit.

Q8. What are the limitations of the blood smear?

Ans: Few limitations of the peripheral smear examination are:[8]

- Experience is required to make technically adequate smears.
- There is a nonuniform distribution of WBCs over the smear, with larger leukocytes concentrated near the edges and lymphocytes scattered throughout. This can lead to erroneous estimation of total leukocyte count (TLC) and differential count.
- There is a nonuniform distribution of RBC over the smear, with small crowded RBCs at the thick edge and large flat RBCs without central pallor at the featured edge.

Q9. Which parasites can be identified on the peripheral smear?

Ans: The microscopic examination of thick and thin peripheral blood smears stained with Giemsa stain is used for detection and identification of *Plasmodium*, *Babesia*, and *Trypanosoma* species and filarial nematodes species (i.e., *Brugia*, *Mansonella*, and *Wuchereria*).[9]

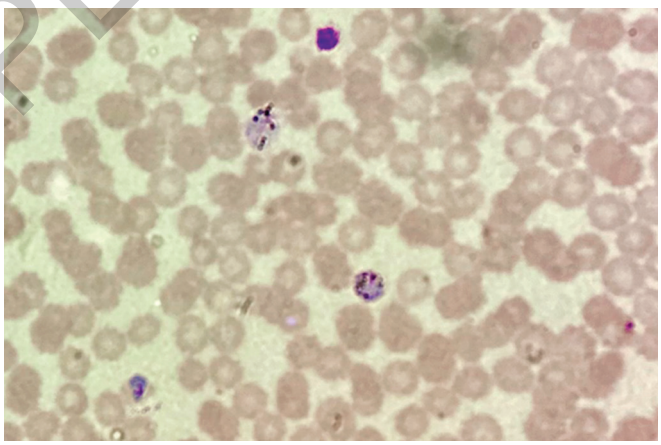

FIG. 6: *Plasmodium vivax* schizont.

Courtesy: Dr Abhishek Purohit.

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Q10. What are commonly used routine and cytochemical stains for peripheral smear?

Ans: Romanowsky stains are used universally for routine staining of blood films.[10] The remarkable property of the Romanowsky dyes of making subtle distinctions in shades of staining, and of staining granules differentially, depends on two components: (i) azure B (trimethyl thionine) and (ii) eosin Y (tetrabromofluorescein). Among the Romanowsky stains in use, Jenner is the simplest and Giemsa is the most complex. Leishman stain, which occupies an intermediate position, is widely used in the routine staining of blood films, although the results are better obtained by combining May–Grünwald–Giemsa, Jenner–Giemsa, and azure B–eosin Y methods. Cytochemical stains are special stains used for staining peripheral blood and bone marrow smears that help in classifying and differentiating different types of leukemias.[11] These stains are cheap, time saving, easy to perform, and yet effective tools in differentiating different types of leukemias. These include:

- Leukocyte alkaline phosphatase (LAP) to differentiate chronic myeloid leukemia (CML)—chronic phase from neutrophilic leukemoid reaction
- Myeloperoxidase stain—to diagnose acute myeloblastic leukemia
- Sudan black B—to diagnose acute myeloblastic leukemia
- Acid phosphatase (ACP)—to diagnose hairy cell leukemia
- Esterases—to diagnose acute monocytic leukemia (AML-M4 or AML-M5)
- Periodic acid–Schiff block positivity is indicative of acute lymphoblastic leukemia.
- Toluidine blue—for rapid detection and species identification of malarial parasites

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Manual of Hematology

Hematology is a diverse branch of medicine that covers both laboratory and clinical aspects of hematology. This book highlights the newer concepts in laboratory hematology in a question and answer format. This book is particularly intended for undergraduate and postgraduate students and fellows. It focuses mainly on the laboratory aspects of benign and malignant hematology and incorporates various techniques used in the diagnosis and management of hematological diseases. Written in an exam-oriented question-answer format, readers will find this book more interesting and focused.



Sanjeev Kumar Sharma is a Director, Hemato-oncology and bone marrow transplant (BMT) expert at BLK-MAX Super Speciality Hospital, New Delhi, and has a great experience in managing patients with benign and malignant blood diseases and has been involved in care of more than 1,000 BMT recipients. He did his MD (Internal Medicine) from University College of Medical Sciences, New Delhi, and DM (Clinical Hematology) from All India Institute of Medical Sciences, New Delhi. He has more than 80 publications in various national and international journals. He has trained many BMT fellows and DNB students. He has authored two well-known books for hematology students and BMT physicians, namely "MCQs in Hematology" and "Basics of Hematopoietic Stem Cell Transplant". This book "*Manual of Hematology*" is also focused on the need of hematology graduates. He has also written a book on a patient's journey through BMT titled "My 32 Days in BMT Ward", and this book has been published in Hindi as well.

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