University of Rwanda

College of medicine and health sciences

School of health sciences

Department of Biomedical laboratory sciences

Year 3

Module: Clinical hematology II

Reg number: 221016345

Laboratory Report

This report highlights almost things that we have covered in the practical sessions of clinical hematology carried out in two consecutive days, on 22nd and 23rdNovember 2023. During these practical sessions, we performed different hematological tests with purpose of understanding the principles behind those tests as well as their interpretation.

Throughout these sessions, we performed the following tests: Erythrocyte sedimentation rate, Reticulocyte count, differential count, screening test of sickle cell, and hemostasis.

1. Erythrocyte sedimentation rate (ESR)

The Erythrocyte Sedimentation Rate (ESR) test, commonly called the Sed rate test, measures how quickly red blood cells settle to the bottom of a test tube in one hour. It can indicate inflammation in the body and help diagnose or monitor certain conditions, including giant cell arteritis, rheumatoid arthritis, and Still's disease. The ESR test is often used alongside other tests to confirm or monitor the progress of an inflammatory condition.

Principle of the test

The Erythrocyte Sedimentation Rate (ESR) test measures how quickly red blood cells settle to the bottom of a test tube. This is done by placing a sample of anticoagulated blood in a vertical tube and letting it sit for one hour. The ESR is measured as the distance in millimeters between the top of the settled red blood cells and the top of the plasma.

There are three stages to the ESR test:

- **Stage of aggregation:** This is the first stage, and it occurs in the first 15 to 20 minutes of the test. During this stage, the red blood cells start to stick together to form rouleaux. Rouleaux are stacks of 4 or more red blood cells caused by increase in plasma proteins such as fibrinogen.
- **Stage of sedimentation:** This is the second stage, and it occurs in the next 30 to 40 minutes of the test. During this stage, the rouleaux settle to the bottom of the test tube at a constant rate.
- **Stage of packing:** This is the third stage, and it occurs in the last 10 minutes of the test. During this stage, the rouleaux pack together tightly, and the rate of sedimentation slows down.

Materials and reagent used.

Materials		**Reagents**
Westergren ESR tube	Tube holders	Anticoagulated blood sample
ESR stand	Lab coats	
Timer	Waste bin	
Aspirator (ESR pipette)		
tourniquet		
Needles		
Gloves		

Procedures of test

- Collection of the venous blood sample in tube containing anticoagulant.
- Aspirate the blood from the tube into Westergren ESR tube.
- Put the Westergren ESR tube containing blood on the ESR stand to make 90^0 and wait for an hour.
- Measured ESR

Result interpretation

ESR test results are measured in millimeters per hour (mm/hr). The normal range for ESR is between 0-20 mm/hr for women and 0-15 mm/hr for men. An elevated ESR may indicate inflammation in the body. However, a high ESR doesn't necessarily mean you have a serious medical condition. Various factors can contribute to a high ESR, including infection, injury, arthritis, cancer, pregnancy, menstruation, and certain medications.

During this practical session, the results fall in normal range because we were using blood of heathy individuals, my classmates.

2. **Reticulocyte count**

Reticulocytes are immature red blood cells that lack a nucleus but still contain some remnants of RNA. Once released from the bone marrow, reticulocytes circulate in the bloodstream for about 1-2 days, during which time they undergo further maturation and gradually shed their remaining RNA remnants and transform into fully functional red blood cells.

Reticulocyte count is a laboratory test that measures the percentage of reticulocytes in the blood. This test is used to monitor and diagnose anemia. There are two main methods for performing a reticulocyte count: automated and manual. In this practical session, we will focus on the manual method.

Principle of the test.

Whole blood is incubated with a supravital stain (such as new methylene blue or brilliant cresyl blue). The supravital stain binds to the ribosomal and residual RNA, as well as the few remaining mitochondria and ferritin granules in young erythrocytes, causing them to clump together and form dark blue filaments and clusters called a **reticulum**.

Smears of this stained mixture are then prepared and examined under a microscope. The number of reticulocytes among 1000 red blood cells is counted. This number is then divided by 10 to obtain the reticulocyte count as a percentage.

Materials and reagents used.

Materials		Reagents
Test tubes	Tube holders	Anticoagulated blood sample
Micropipette and their tips		Supravital stain (brilliant cresyl blue)
Timer		
Microscope		
Slides		
Needles		
Gloves		

Procedures of the test.

- Mix the equal volumes of supravital stain and anticoagulated blood sample. In this case, we had used 500µl of stain and mixed with 500µl of the sample.
- We mixed gently and incubated for 30 minutes at room temperature.
- We prepared the thin smear on two slides and then chosen one best smear.
- Allowed it to dry and after drying, we put the drop of immersion oil on that slide.
- We then, observed under microscope by using the objective lens of 100 times.

Result interpretation and reporting.

To count the number of reticulocytes, start by counting the number of red blood cells (RBCs) in each microscopic field. Continue counting until you reach a total of 1000 RBCs. Then, count the number of reticulocytes within those 1000 RBCs.

To report the reticulocyte count, you can use two methods: relative (percentage) or absolute value.

- **Relative (percentage) method:** Take the number of reticulocytes counted and divide it by 10. The resulting number represents the percentage of reticulocytes in the blood sample.

- **Absolute value method:** Report the total number of reticulocytes counted per 1000 RBCs.

When observing reticulocytes under the microscope, you will notice that they appear as RBCs with dark blue-violet dots inside. These dots are composed of ribosomal RNA.

Normal ranges:

- Infant at birth: 2-5 %
- Child and adult: 0.5-2.5 %

The reticulocyte count is a good indicator that shows how the bone marrow are performing. High level of reticulocyte indicates that case of hemolytic anemia, and the low number of reticulocytes indicates a plastic anemia.

High level of reticulocyte is called **reticulocytosis** while low level of reticulocyte is also called **reticulocytopenia**.

3. **Differential count**

A differential count, also known as a white blood cell (WBC) differential, is a blood test that measures the proportion of different types of white blood cells in your blood. White blood cells are crucial components of your immune system, playing a vital role in defending your body against infections. The five main types of white blood cells are **lymphocytes**, **neutrophils**, **basophils**, and **eosinophils**.

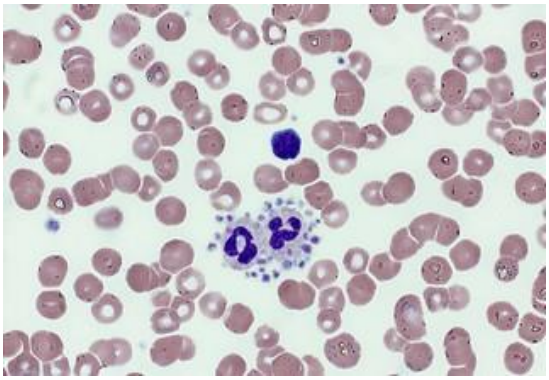
Principles of the test

The principle of a differential count, also known as a white blood cell (WBC) differential, is based on the distinct microscopic features of various white blood cell (WBC) types. A blood smear is prepared and stained with specific dyes, allowing for the visual identification of individual WBCs under a microscope.

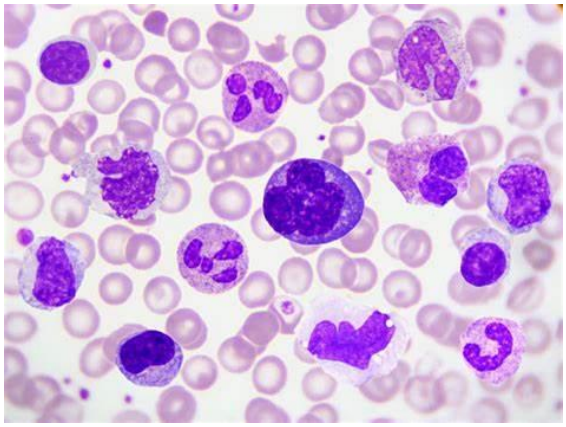
It carried out in two steps:

- **Fixation and staining with MGG:** May-Grünwald solution is a mixture of methanol, eosin, and methylene blue used to fix and stain blood smears for microscopic

examination. Methanol acts as a fixative, preserving the cellular morphology. Eosin is an acidic dye that stains acidic cellular components, primarily DNA and RNA, in a pink or red color. Methylene blue, a basic dye, stains basic cellular components, such as cytoplasmic proteins, in a blue color.



- **Differentiation with Giemsa:** Giemsa solution, a combination of methanol, eosin, and azure dyes, is employed to further differentiate and enhance the staining of acidic and basic cellular components in blood smears. Methanol aids in the differentiation process by facilitating the selective staining of various cellular structures. Eosin, an acidic dye, imparts a pink or red hue to acidic cellular components, primarily DNA and RNA. Azure dyes, a group of basic dyes, further enhance the staining of basic cellular components, particularly cytoplasmic proteins and ribosomes, providing a deeper blue coloration.



Materials and reagents used.

Materials		Reagents
Gloves	Manual blood cell counter	Anticoagulated blood sample
Micropipette and their tips	Staining rack	May-Grunwald solution
Timer		Giemsa solution
Microscope		Immersion oil
Slides		Tap water
Needles		

Procedures of the test

The following are the procedures that are used in differential count of blood cells:

- We prepare peripheral blood thin smear.
- Allow it to dry and then, put it on staining rack to start staining.
- Put May-Grunwald solution on the thin smear slide for 5 minutes for fixation.
- Rinse the slide with tap water.
- Put Giemsa solution on the slide and wait for 20 minutes.
- Rinse with water and allow it to dry.
- Put a drop of immersion oil on the slide and then observe and count under light microscope by using objective lens of 100 times.
- Report your result.

Result reporting and interpretation

Determining the differential count involves manually or using automated machines to count the different types of white blood cells (WBCs) in a blood smear. In our practical session, we employed the manual method, meticulously counting each WBC in each microscopic field. We continued counting until reaching a total of 100 cells and recorded the number of each WBC type observed.

Variations in WBC counts can signal underlying health issues. An elevated neutrophil count may indicate a bacterial infection, while an increased lymphocyte count suggests a viral infection. Conversely, a reduced WBC count could be indicative of conditions like aplastic anemia.

Differential white blood cells count
(normal adult)

	Absolute Values	Percentage.
Neutrophil	2.0-7.0 x10^9/L	40-75 %
Lymphocytes	1.0-3.0 x10^9/L	20-45 %
Monocytes	0.2-1.0 x10^9/L	2-10 %
Eosinophil	0.02-0.5 x10^9/L	1-6 %
Basophile	0.02-0.1 x10^9/L	0-2 %

Screening test for sickle cell anemia.

Sickle cell disease (SCD) is an inherited group of blood disorders that affect hemoglobin, the protein in red blood cells that carries oxygen throughout the body. In individuals with SCD, hemoglobin is abnormal, causing red blood cells to adopt a sickle-like or crescent-shaped appearance instead of their typical round shape. This abnormal shape makes the cells less flexible and more prone to sticking together, obstructing blood flow.

The sickle cell trait arises from a single amino acid substitution in the β-globin chain of hemoglobin, specifically at the sixth position, where **glutamic acid** is replaced by **valine**. With Hb-C, valine is replaced by lysine amino acid.

This test of sickle cell is performed by using sodium metabisulphite solution which reduces the affinity of hemoglobin to bind to bind to Oxygen.

In the presence of low oxygen tension, sodium metabisulfite induces the polymerization of hemoglobin S (HbS) into elongated fibers, causing the red blood cells to sickle.

Principle of sodium metabisulphite

The sodium metabisulfite test utilizes the property of HbS, the abnormal hemoglobin in sickle cell disease, to form elongated fibers under low oxygen conditions. Sodium metabisulfite serves

as a reducing agent, establishing an environment with low oxygen tension that facilitates HbS polymerization.

Materials and reagents used.

Materials		Reagents
Gloves	tubes	Anticoagulated blood sample
Petri dishes	coverslips	Sodium metabisulphite solution
Timer	Pipettes	
Microscope	Tissue paper	
Slides		
Needles		

Procedures of the test

The following are the procedures that are followed during this test of sodium metabisulphite solution.

- Collect the venous blood and prepare the sodium metabisulphite solution.
- Mix the equal volume of both blood sample and Sodium metabisulphite solution.
- Mix the solution gently and incubate for 30 minutes at room temperature.
- Take a drop of solution and prepare wet mount.
- Put slides in covered Petri dishes.
- Observe under microscope.
- Report your results.

Result interpretation

Upon microscopic examination, individuals with sickle cell trait exhibit red blood cells with a characteristic moon-like shape, suggesting the presence of HbS. However, this test is not definitive and should be corroborated by other confirmatory tests, such as hemoglobin electrophoresis.

4. HEMOSTASIS

Hemostasis is the body's normal process of stopping bleeding. It is a complex process that involves multiple steps, including:

- **Vasoconstriction:** This is the narrowing of blood vessels. It helps to reduce blood flow to the injured area.
- **Platelet adhesion:** Platelets are sticky blood cells that clump together at the site of an injury. This helps to form a plug that stops bleeding.
- **Coagulation:** This is the formation of a blood clot. A blood clot is a gel-like substance that helps to seal off the injured blood vessel.
- **Fibrinolysis:** This is the breakdown of a blood clot. It is important to prevent blood clots from forming in the wrong places, such as in the arteries or veins.

The hemostasis process encompasses two phases: primary hemostasis and secondary hemostasis. During the practical session, we focused on primary hemostasis.

Specifically, we conducted the bleeding time test, which measures the time it takes for an individual to bleed following an injury or during surgery. This test incorporates both **activated partial thromboplastin time (aPTT)** and **prothrombin time (PT)**.

Principle of the test

The bleeding time test rests on the principle that the formation of a platelet plug at the injury site is a crucial component of primary hemostasis, the initial phase of hemostasis. A minor skin incision triggers the release of platelets from the exposed blood vessels.

These platelets adhere to the damaged tissue and coalesce to form a platelet plug, effectively sealing the wound and halting further bleeding. The duration of bleeding until cessation reflects the efficiency of platelet adhesion, activation, and aggregation, all of which are essential elements of primary hemostasis.

Materials used in the test.

Lancet, timer, blotting paper, alcohol, bandages, gloves, sharp container, marking pen, ruler or measuring tape.

Procedures of the test

The procedures that are used in bleeding time test include the followings:

- **Patient preparation**: Inform the patient about the purpose of the test and obtain their informed consent.
- **Incision**: Wear sterile gloves to prevent cross-contamination. Clean the selected site with an alcohol swab to disinfect the skin. Using a sterile, disposable lancet with a standardized blade size, make a precise incision 10mm long and 1mm deep.
- **Blotting:** Immediately start a timer. Gently touch the tip of a piece of filter paper to the incision every 30 seconds without applying any pressure. Continue blotting until the bleeding has completely stopped and the filter paper remains free of blood for two consecutive 30-second intervals.
- **Recording Results:** Stop the timer and record the time it took for bleeding to stop. Note any observations during the test, such as excessive bleeding or difficulty in stopping the bleeding.
- **Bandaging:** Cover the incision with an adhesive bandage to prevent further bleeding.
- **Disposal of Sharps**: Safely dispose of the used lancet in a designated sharps container to prevent accidental injuries.
- **Hand Hygiene:** Wash your hands thoroughly with soap and water after removing the gloves.

Result reporting and interpretation

Once the bleeding has ceased, record the time elapsed during the bleeding episode. The normal bleeding time reference range is less than seven minutes.

Conclusion

This concludes the practical session, during which the intended objectives were successfully achieved. Each participant demonstrated the ability to independently perform the test series and interpret their results in accordance with the laboratory procedures.