STANDARD OPERATING PROCEDURES
HEMATOLOGY

Province Government
Ministry of Health
Province Public Health Laboratory
Koshi Province
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Title: Sample Collection/ Phlebotomy		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1.1 Scope and Application

Phlebotomy is the process of making a puncture in a vein, usually in the ante-cubital vein of arm, with a needle or cannula for the purpose of drawing blood. The procedure itself is known as a venipuncture. A person who performs a phlebotomy is called a phlebotomist.

PURPOSE:

- To perform various tests (Biochemical, hematological, microbiological) in order to access the current health state.
- To monitor the therapeutic treatment including medications, nutrition or chemotherapy.
- To collect blood for Blood banking/ Transfusion.

1.2 Responsibility:

It is the responsibility of the registered laboratory personnel/ phlebotomist to collect the sample.

1.3 Principle

To obtain blood samples by venipuncture.

1.4 Materials and Reagents

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

SN.	Materials and Equipment
1.	Vaccutainer set
2.	Evacuated blood collection (Vaccutainer) tubes (e.g. Tube for separating serum/Serum separator **clot activator**)
3.	Evacuated blood collection (Vaccutainer) tubes (e.g. **Lavender top** tube with EDTA)
4.	3.2% Tri-sodium citrate (Vaccutainer) tubes (e.g. **Blue top** tube)
5.	Eppendorf tubes for serum storage 3 per subjects
6.	Tourniquet / Arm band
7.	Alcohol wipes (70%) isopropyl alcohol
8.	Gauze sponges or cotton ball for application to site from which needle has been withdrawn
9.	Gloves (non-latex recommended) worn to protect subject and phlebotomist
10.	Syringes that may be used in place of evacuated collection tubes in certain circumstances (3,5 or 10 mL Syringe with needle)
11.	Stationary items (Labels, Permanent marker, Pen, pencil etc.)

SITE:

1.) Median ante-cubital vein
2.) Cephalic vein
3.) Basilic vein
4.) OR any other palpable vein

1.5 Procedure:

Step 1. Assemble Materials Required

Collect all the Materials needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. The equipment required includes:

- a supply of laboratory sample tubes, which should be stored dry and upright in a rack; blood can be collected in:
  - sterile glass or plastic tubes with rubber caps (the choice of tube will depend on what is agreed with the laboratory);
  - Vacuum-extraction blood tubes; or
  - glass tubes with screw caps;
- a sterile glass or bleeding pack (collapsible) if large quantities of blood are to be collected;
- well-fitting, non-sterile gloves;
- an assortment of blood-sampling devices (safety-engineered devices or needles and syringes, see below), of different sizes;
- a tourniquet;
- alcohol hand rub;
- 70% alcohol swabs for skin disinfection;
- gauze or cotton-wool ball to be applied over puncture site;
- laboratory specimen labels;
- writing equipment;
- laboratory forms;
- leak-proof transportation bags and containers;
- a puncture-resistant sharps container.

Ensure that the rack containing the sample tubes is close to you, the health worker, but away from the patient, to avoid it being accidentally tipped over.

Step 2. Identify and prepare the patient

Where the patient is adult and conscious follow the steps outlined below.

- Introduce yourself to the patient, and ask the patient to state their full name.
- Check that the laboratory form matches the patient's identity (i.e. match the patient's details with the laboratory form, to ensure accurate identification).
- Ask whether the patient has allergies, phobias or has ever fainted during previous injections or blood draws.
- If the patient is anxious or afraid, reassure the person and ask what would make them more comfortable.
- Make the patient comfortable in a supine position (if possible).
- Place a clean paper or towel under the patient's arm.
- Discuss the test to be performed and obtain verbal consent. The patient has a right to refuse a test at any time before the blood sampling, so it is important to ensure that the patient has understood the procedure.

Step 3. Select the site

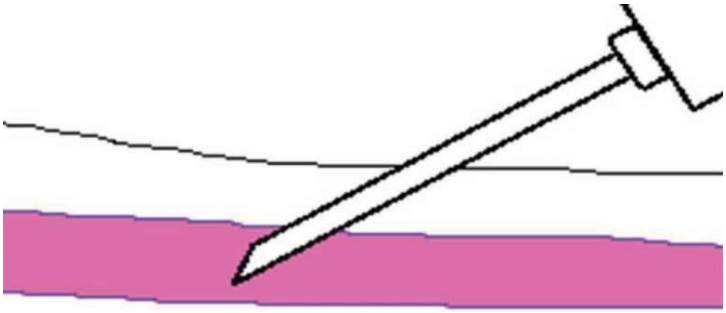
General

- Extend the patient's arm and inspect the ante-cubital vein.
- Locate a vein of a good size that is visible, straight and clear. The median cubital vein lies between muscles and is usually the most easy to puncture. Under the basilic vein runs an artery and a nerve, so puncturing here runs the risk of damaging the nerve or artery and is usually more painful. DO NOT insert the needle where veins are diverting, because this increases the chance of a hematoma.

- If superficial veins are not readily apparent, you can force blood into the vein by massaging the arm from wrist to elbow, tap the site with index and second finger, apply a warm, damp washcloth to the site for 5 minutes, or lower the extremity over the bedside to allow the veins to fill.
  - ➢ Certain areas are to be avoided when choosing a site:
- Extensive scars from burns and surgery - it is difficult to puncture the scar tissue and obtain a specimen.
- The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.
- Hematoma - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
- Intravenous therapy (IV) / blood transfusions - fluid may dilute the specimen, so collect from the opposite arm if possible. Otherwise, satisfactory samples may be drawn below the IV by following these procedures:
  - Turn off the IV for at least 2 minutes before venipuncture.
  - Apply the tourniquet below the IV site. Select a vein other than the one with the IV.
  - Perform the venipuncture. Draw 5 ml of blood and discard before drawing the specimen tubes for testing.
- Lines - Drawing from an intravenous line may avoid a difficult venipuncture, but introduces problems. The line must be flushed first. When using a syringe inserted into the line, blood must be withdrawn slowly to avoid hemolysis.
- Cannula/fistula/heparin lock - hospitals have special policies regarding these devices. In general, blood should not be drawn from an arm with a fistula or cannula without consulting the attending physician.
- Edematous extremities - tissue fluid accumulation alters test results.

Step 4. PERFORMANCE OF A VENIPUNCTURE:

- Approach the patient in a friendly, calm manner. Provide for their comfort as much as possible, and gain the patient's cooperation.
- Identify the patient correctly.
- Properly fill out appropriate requisition forms, indicating the test(s) ordered.
- Verify the patient's condition. Fasting, dietary restrictions, medications, timing, and medical treatment are all of concern and should be noted on the lab requisition.
- Check for any allergies to antiseptics, adhesives, or latex by observing for armbands and/or by asking the patient.
- Position the patient. The patient should sit in a chair, lie down or sit up in bed. Hyperextend the patient's arm.
- Apply the tourniquet 3-4 inches above the selected puncture site. Do not place too tightly or leave on more than 2 minutes (and no more than a minute to avoid increasing risk for hemoconcentration). Wait 2 minutes before reapplying the tourniquet.
- The patient should make a fist without pumping the hand.
- Select the venipuncture site.
- Prepare the patient's arm using an alcohol prep. Cleanse in a circular fashion, beginning at the site and working outward. Allow to air dry.
- Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. The needle should form a 15 to 30 degree angle with the surface of the arm. Swiftly insert the needle through the skin and into the lumen of the vein. Avoid trauma and excessive probing.

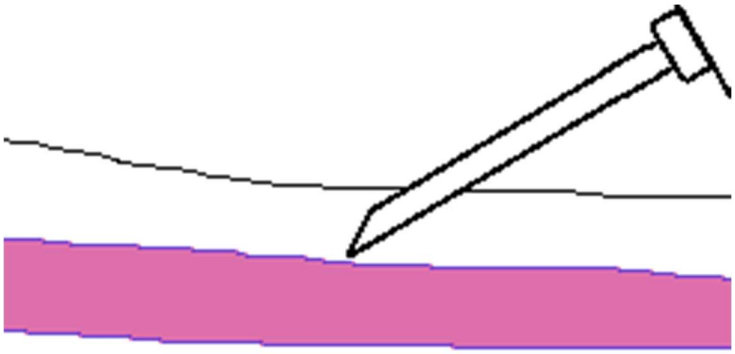


- When the last tube to be drawn is filling, remove the tourniquet.
- Remove the needle from the patient's arm using a swift backward motion.
- Press down on the gauze once the needle is out of the arm, applying adequate pressure to avoid formation of a hematoma.
- Dispose of contaminated materials/supplies in designated containers.
- Mix and label all appropriate tubes at the patient bedside.
- Deliver specimens promptly to the laboratory.

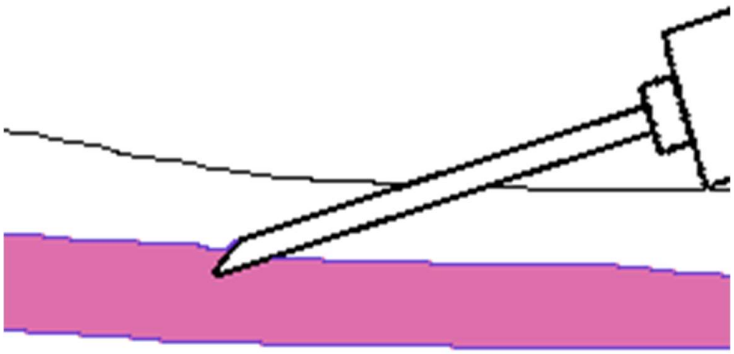
TROUBLESHOOTING GUIDELINES:

IF AN INCOMPLETE COLLECTION OR NO BLOOD IS OBTAINED:

- Change the position of the needle. Move it forward (it may not be in the lumen)



- or move it backward (it may have penetrated too far).
- Adjust the angle (the bevel may be against the vein wall).



- Loosen the tourniquet. It may be obstructing blood flow.
- Try another tube. Use a smaller tube with fewer vacuums. There may be no vacuum in the tube being used.
- Re-anchor the vein. Veins sometimes roll away from the point of the needle and puncture site.
- Have the patient make a fist and flex the arm, which helps engorge muscles to fill veins.
- Pre-warm the region of the vein to reduce vasoconstriction and increase blood flow.
- Have the patient drink fluids if dehydrated.

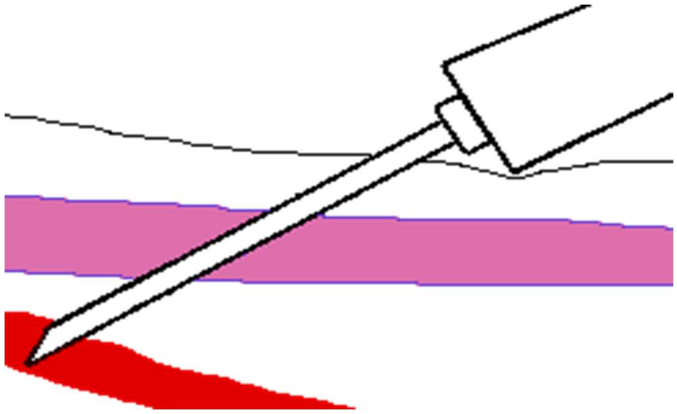
IF BLOOD STOPS FLOWING INTO THE TUBE:

- The vein may have collapsed, resecure the tourniquet to increase venous filling. If this is not successful, remove the needle, take care of the puncture site, and redraw.

- The needle may have pulled out of the vein when switching tubes. Hold equipment firmly and place fingers against patient's arm, using the flange for leverage when withdrawing and inserting tubes.

PROBLEMS OTHER THAN AN INCOMPLETE COLLECTION:

- A hematoma forms under the skin adjacent to the puncture site - release the tourniquet immediately and withdraw the needle. Apply firm pressure.

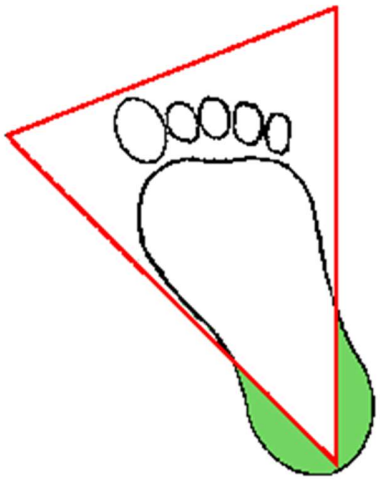


Hematoma formation is a problem in older patients.

- The blood is bright red (arterial) rather than venous. Apply firm pressure for more than 5 minutes.

BLOOD COLLECTION ON BABIES:

- The recommended location for blood collection on a newborn baby or infant is the heel. The diagram below indicates in green the proper area to use for heel punctures for blood collection:



- Prewarming the infant's heel (42 C for 3 to 5 minutes) is important to obtain capillary blood gas samples and warming also greatly increases the flow of blood for collection of other specimens. However, do not use too high a temperature warmer, because baby's skin is thin and susceptible to thermal injury.
- Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry cotton sponge. Hold the baby's foot firmly to avoid sudden movement.
- Using a sterile blood lancet, puncture the side of the heel in the appropriate regions shown above in green. Do not use the central portion of the heel because you might injure the underlying bone, which is close to the skin surface. Do not use a previous puncture site. Make the cut across the heel print lines so that a drop of blood can well up and not run down along the lines.
- Wipe away the first drop of blood with a piece of clean, dry cotton. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure or heavy massaging because the blood may become diluted with tissue fluid.
- Fill the capillary tube(s) or micro collection device(s) as needed.
- When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped.
- Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles. Remove your gloves and wash your hands.

Step 5. Fill the laboratory sample tubes

- When obtaining multiple tubes of blood, use evacuated tubes with a needle and tube holder. This system allows the tubes to be filled directly. If this system is not available, use a syringe or winged needle set instead.

- If a syringe or winged needle set is used, best practice is to place the tube into a rack before filling the tube. To prevent needle-sticks, use one hand to fill the tube or use a needle shield between the needle and the hand holding the tube.
- Pierce the stopper on the tube with the needle directly above the tube using slow, steady pressure. Do not press the syringe plunger because additional pressure increases the risk of haemolysis.
- Where possible, keep the tubes in a rack and move the rack towards you. Inject downwards into the appropriate colored stopper. DO NOT remove the stopper because it will release the vacuum.
- If the sample tube does not have a rubber stopper, inject extremely slowly into the tube as minimizing the pressure and velocity used to transfer the specimen reduces the risk of haemolysis. DO NOT recap and remove the needle.
- Before dispatch, invert the tubes containing additives for the required number of times (as specified by the local laboratory).

Step 6. Draw samples in the correct order

Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw for plastic vaccutainer tubes is:

S.N	COLLECTION TUBE	ADDITIVE	INVERSION	DETERMINATION
1.	Blood Culture	Blood: Broth (1:10)	8-10 Times	Blood Culture
2.	PT Tube (Blue Cap)	Tri-sodium citrate (3.2%) Blood: Citrate (1:9)	3-4 Times	PT, APTT and other coagulation profile tests
3.	Gel Tube (Yellow Cap)	Clot Activation Gel Tube	5 Times	Biochemistry, Serology, Immunoassay
4.	Lavender Purple Cap	EDTA	8-10 Times	Hematological Tests, HbA1c
5.	Grey Cap	Sodium Fluoride	8-10 Times	Glucose
6.	Black Cap	Tri-sodium Citrate 3.8% Blood:Citrate 1:4	3-4 Times	ESR

Draw blood collection tubes in the correct order, to avoid cross-contamination of additives between tubes. As colour coding and tube additives may vary, verify recommendations with local laboratories.

ADDITIONAL CONSIDERATIONS:

To prevent a hematoma:

- Puncture only the uppermost wall of the vein
- Remove the tourniquet before removing the needle
- Use the major superficial veins
- Make sure the needle fully penetrates the upper most wall of the vein. (Partial penetration may allow blood to leak into the soft tissue surrounding the vein by way of the needle bevel)
- Apply pressure to the venipuncture site

To prevent hemolysis (which can interfere with many tests):

- Mix tubes with anticoagulant additives gently 5-10 times

- Avoid drawing blood from a hematoma
- Avoid drawing the plunger back too forcefully, if using a needle and syringe, or too small a needle, and avoid frothing
- of the sample
- Make sure the venipuncture site is dry
- Avoid a probing, traumatic venipuncture
- Avoid prolonged tourniquet application or fist clenching.

Indwelling Lines or Catheters:

- Potential source of test error
- Most lines are flushed with a solution of heparin to reduce the risk of thrombosis
- Discard a sample at least three times the volume of the line before a specimen is obtained for analysis

Hemoconcentration: An increased concentration of larger molecules and formed elements in the blood may be due to several factors:

- Prolonged tourniquet application (no more than 1 minute)
- Massaging, squeezing, or probing a site
- Long-term IV therapy
- Sclerosed or occluded veins

Prolonged Tourniquet Application:

- Primary effect is hemoconcentration of non-filterable elements (i.e. proteins). The hydrostatic pressure causes some water and filterable elements to leave the extracellular space.
- Significant increases can be found in total protein, aspartate aminotransferase (AST), total lipids, cholesterol, iron
- Affects packed cell volume and other cellular elements
- Hemolysis may occur, with pseudohyperkalemia.

Patient Preparation Factors:

- Therapeutic Drug Monitoring: different pharmacologic agents have patterns of administration, body distribution, metabolism, and elimination that affect the drug concentration as measured in the blood. Many drugs will have "peak" and "trough" levels that vary according to dosage levels and intervals. Check for timing instructions for drawing the appropriate samples.
- Effects of Exercise: Muscular activity has both transient and longer lasting effects. The creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and platelet count may increase.
- Stress: May cause transient elevation in white blood cells (WBC's) and elevated adrenal hormone values (cortisol and catecholamines). Anxiety that results in hyperventilation may cause acid-base imbalances, and increased lactate.
- Diurnal Rhythms: Diurnal rhythms are body fluid and analyte fluctuations during the day. For example, serum cortisol levels are highest in early morning but are decreased in the afternoon. Serum iron levels tend to drop during the day. You must check the timing of these variations for the desired collection point.
- Posture: Postural changes (supine to sitting etc.) are known to vary lab results of some analytes. Certain larger molecules are not filterable into the tissue, therefore they are more concentrated in the blood. Enzymes, proteins, lipids, iron, and calcium are significantly increased with changes in position.
- Other Factors: Age, gender, and pregnancy have an influence on laboratory testing. Normal reference ranges are often noted according to age.

PERFORMANCE OF A FINGERSTICK:

- Follow the procedure as outlined above for greeting and identifying the patient. As always, properly fill out appropriate requisition forms, indicating the test(s) ordered.

- Verify the patient's condition. Fasting, dietary restrictions, medications, timing, and medical treatment are all of concern and should be noted on the lab requisition.
- Position the patient. The patient should sit in a chair, lie down or sit up in bed. Hyperextend the patient's arm.
- The best locations for finger sticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
- Using a sterile lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
- Wipe away the first drop of blood, which tends to contain excess tissue fluid.
- Collect drops of blood into the collection device by gently massaging the finger. Avoid excessive pressure that may squeeze tissue fluid into the drop of blood.
- Cap, rotate and invert the collection device to mix the blood collected.
- Have the patient hold a small gauze pad over the puncture site for a couple of minutes to stop the bleeding.
- Dispose of contaminated materials/supplies in designated containers.
- Label all appropriate tubes at the patient bedside.

Deliver specimens promptly to the laboratory.

Step 7. Clean contaminated surfaces and complete patient procedure

- Discard the used needle and syringe or blood sampling device into a puncture-resistant sharps container.
- Check the label and forms for accuracy. The label should be clearly written with the information required by the laboratory, which is typically the patient's first and last names, file number, and the date and time when the blood was taken.
- Discard used items into the appropriate category of waste. Items used for phlebotomy that would not release a drop of blood if squeezed (e.g. gloves) may be discarded in the general waste, unless local regulations state otherwise.
- Perform hand hygiene again, as described above.
- Recheck the labels on the tubes and the forms before dispatch.

- Inform the patient when the procedure is over.
- Ask the patient or donor how they are feeling. Check the insertion site to verify that it is not bleeding.

Step 8. Prepare samples for transportation

- Pack laboratory samples safely in a plastic leak-proof bag with an outside compartment for the laboratory request form. Placing the requisition on the outside helps avoid contamination.
- If there are multiple tubes, place them in a rack or padded holder to avoid breakage during transportation.

Step 9. Clean up spills of blood or body fluids

If blood spillage has occurred (e.g. because of a laboratory sample breaking in the phlebotomy area or during transportation, or excessive bleeding during the procedure), clean it up. An example of a safe procedure is given below.

- Put on gloves and a gown or apron if contamination or bleaching of a uniform is likely in a large spill.
- Mop up liquid from large spills using paper towels, and place them into the infectious waste.
- Remove as much blood as possible with wet cloths before disinfecting.
- Assess the surface to see whether it will be damaged by a bleach and water solution.
- For cement, metal and other surfaces that can tolerate a stronger bleach solution, flood the area with an approximately 5000 parts per million (ppm) solution of sodium hypochlorite (1:10 dilution of a 5.25% chlorine bleach to water). This is the preferred concentration for large spills. Leave the area wet for 10 minutes.
- For surfaces that may be corroded or discolored by strong bleach, clean carefully to remove all visible stains. Make a weaker solution and leave it in contact for a longer period of time. For example, an approximately 525 ppm solution (1:100 dilution of 5.25% bleach) is effective.
- Prepare bleach solution fresh daily and keep it in a closed container because it degrades over time and in contact with the sun.

If a person was exposed to blood through non-intact skin, mucous membranes or a puncture wound, complete an incident report, as described in *WHO best practices for injections and related procedures toolkit*. For transportation of blood samples outside a hospital, equip the transportation vehicle with a blood spillage kit.

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 11 of 12
Title: Blood Sample Handling and Processing		Approved By: Director
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Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and application:

A sample received in laboratory requires proper handling as it may be hazardous to the technical staffs for which a proper handling step should be followed. Similarly, some hematological tests require serum or plasma as samples for which we need to centrifuge the clotted blood or citrated whole blood.

Purpose

- To handle blood samples in laboratory
- To properly centrifuge the samples

2. Responsibility: It is the responsibility of the registered laboratory personnel to properly separate the samples.

3. Specimen Type:

For all types of specimens

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8 °C	Upto 72 hrs (3 days)
Whole blood	Room temperature	Upto 24hrs

5. Principle

- To handle samples carefully

6. Materials and reagents:

- Centrifuge
- EDTA OR Citrated Blood Samples

7. Procedure:

Pre-centrifugation Handling

The first critical step in the lab testing process, after obtaining the sample, is the preparation of the blood samples. Specimen integrity can be maintained by following some basic handling processes:

- Fill tubes to the stated draw volume to ensure the proper blood-to-additive ratio. Allow the tubes to fill until the vacuum is exhausted and blood flow ceases.
- Vaccutainer tubes or blood collection tube should be stored at 4-25°C (39-77°F).
- Tubes should not be used beyond the designated expiration date.

- Mix all gel barrier and additive tubes by gentle inversion 5 to10 times immediately after the draw. This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
- Serum separator tubes should clot for a full 30 minutes in a vertical position prior to centrifugation. Short clotting times can result in fibrin formation, which may interfere with complete gel barrier formation.

Blood Sample Centrifugation

It is recommended that serum be physically separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection.

- Complete gel barrier formation (gel barrier tubes) is time, temperature and G-force dependent. The uniformity of the barrier is time dependent; an incomplete barrier could result from shortened centrifugation times.
- In general, for a horizontal, swing-bucket centrifuge, the recommended spin time is 10 minutes and for a fixed-angle centrifuge, the recommended spin time is 15 minutes at 2500 to 3000rpm.
- NOTE: Gel flow may be impeded if chilled before or after centrifugation.
- Tubes should remain closed at all times during the centrifugation process.
- Place the closed tubes in the centrifuge as a “balanced load” noting the following:
    - Opposing tube holders must be identical and contain the same cushion or none at all.
    - Opposing tube holders must be empty or loaded with equally weighted samples (tubes of the same size and equal in fill).
    - If an odd number of samples is to be spun, fill a tube with water to match the weight of the unpaired sample and place it across from this sample.

Centrifuge Safety

- Interference with an activated centrifuge by an impatient employee can result in bodily injury in the form of direct trauma or aerosolization of hazardous droplets.
- Centrifuges must never be operated without a cover in place.
- Uncovered specimen tubes must not be centrifuged.
- Centrifuges must never be slowed down or stopped by grasping part(s) of the device with your hand or by applying another object against the rotating equipment.
- Be sure the centrifuge is appropriately balanced before activating. If an abnormal noise, vibration, or sound is noted while the centrifuge is in operation, immediately stop the unit (turn off the switch) and check for a possible load imbalance.
- Clean the centrifuge daily with a disinfectant and paper towel. Broken tubes or liquid spills must be cleaned immediately.

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 13 of 15
Title: Haemoglobin Estimation		Approved By: Director
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Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for estimation of Haemoglobin Concentration in whole blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

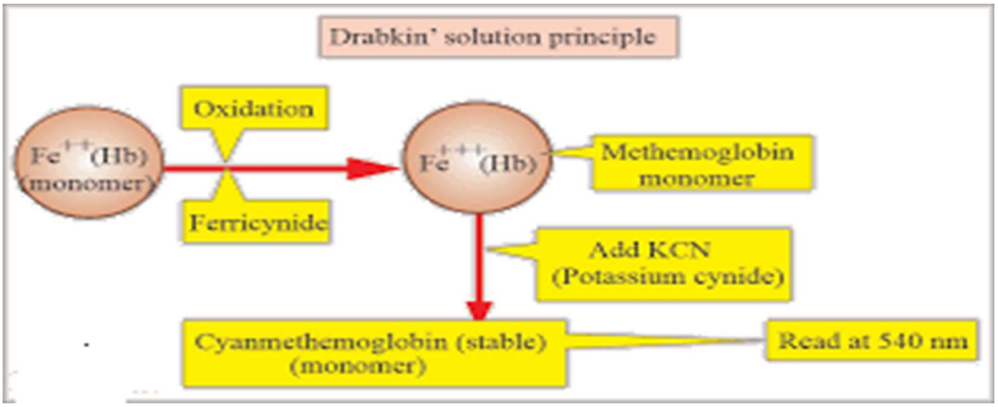
EDTA Anticoagulated Blood

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8 °C	Upto 72 hrs (3 days)
Whole blood	Room temperature	Upto 24hrs

5. Principle

When blood is mixed with Drabkin's reagent containing potassium cyanide and potassium ferricyanide, Hb reacts with ferricyanide to form methemoglobin which is converted to stable cyanmethemoglobin (HiCN) by the cyanide & the intensity of colour is measured in colorimeter at 540nm (green filter).



6. Materials and Reagents

- Pipette
- Test tubes (15X125mm)
- Test tube rack
- Colorimeter
- Drabkin's Solution

- Drabkin's solution contains:
  - Potassium ferricyanide = 200 mg
  - Potassium cyanide = 50 mg
  - Potassium dihydrogen phosphate = 140 mg
  - Non-ionic detergent = 1 ml
  - Distal water = Make up to 1000 ml (1 L)
- Hb Standard (15gm/dl)(Commercially available)

7. Test Procedure

1. Take 3 large size test tubes and label 'Test', 'Standard' and 'Blank' on each tube.
2. Add 5ml of Drabkin's solution in each tube.
3. Then pipette 20µl of well mixed anticoagulated blood in tube labelled 'Test' and mix well, follow the table below
4. Rinse the pipette.
5. Let the mixture stand for 10 minutes at R.T. for total reaction to occur.
6. Read the O.D. at 540 nm against blank (Drabkin's Solution) in colorimeter.
7. Test the haemoglobin standard in similar manner like the test and record the Optical densities(O.D.) of both test and Standard
8. Get the result by using the following calculation.

	Blank	Standard	Test
Sample	-	-	20ul
Standard	-	5ml	-
Drabkin's reagent	5ml	-	5ml

8. Calculation:

$$Concentration\ of\ Hb = \frac{OD\ of\ Test}{OD\ of\ Std} \times concentration\ of\ std$$

9. Biological Reference:

Male: 13-17.5 g/dl

Female: 12-16 g/dl

Neonatal: 14.5-24.5 g/dl

Children (up to 1 year): 11.0-13.0 g/dl

Children (10-12 years): 11.5-14.5g/dl

10. Critical Value:

High Value	Low Value
>20gm/dl	<7gm/dl

11. Limitation

Lipemic blood, presence of HbS or, HbC, high leukocyte count may result in turbidity resulting in erroneous values.

12. Interference

Hemoglobin is quantified based on its absorption characteristics.Conditions such as hyperlipidemias, hyperbilirubinemia, a very high white blood cell count, and high serum protein can interfere with this measurement and result in falsely elevated haemogobin values.

13. Quality Control

Test standard as like the patient's sample and check for precision and accuracy of the test results before dispatching the report to the patients.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

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https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 16 of 18
Title: Total Leukocyte Count		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for calculation of total leukocyte count in the whole blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

EDTA Anticoagulated Blood

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8 ^{o}C	Up to 72 hrs (3 days)
Whole blood	Room temperature	Up to 24hrs

5. Principle

The components of Turk's fluid (WBC diluting fluid) like glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leukocytes. The blood specimen is diluted 1:20 with the WBC diluting fluid and the cells are counted under low power of the microscope by using an Improved Neubauer counting chamber. The number of cells in undiluted blood is reported as Cells per Cumm of whole blood.

6. Materials and Reagents

- Pipette
- Test tubes
- Test tube rack
- Improved Neubauer Counting Chamber (hemocytometer)
- Counting chamber cover glass(25.4mm x76.2mm)
- Microscope.
- Cell counter
- WBC diluting fluid (Turk's Fluid)

  Composition:

  1% Gentian Violet 1ml

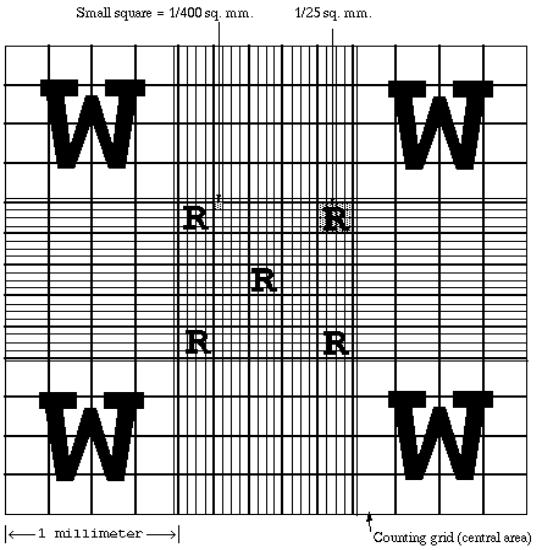
  Glacial acetic acid 1.5ml

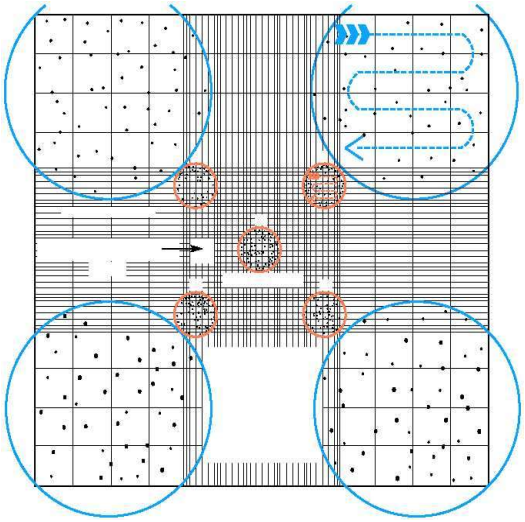
  Distilled water upto 100 ml

Thymol 1pinch

7. Procedures:

1. With the help of a well Calibrated automatic pipette measure 380 µl of WBC diluting fluid & dispense it into a tube.
2. Add 20 µl of well-mixed EDTA anticoagulated venous blood.
3. After adequate mixing, counting chamber is charged with the mixture.
4. Leave the chamber undisturbed for 2 min to allow time for the white blood cells to settle.
5. After 2 min. WBCs are counted in 4 corner squares of the counting chamber i.e. W1,W2,W3,W4. So, the area counted becomes $4mm^2$.
6. Then total number of WBCs are counted as below





8.Calculation:

- Number of cells counted = N
- Counted area = (A) = $4mm^2$
- Chamber depth = (d) = 1/10 mm
  i.e. depth factor = 10
- Dilution = 1:20
- Dilution factor =20

WBC count Formula:

$$WBC/Cumm = \frac{N \times dilution\ factor \times depth\ factor}{\text{Area counted}}$$

$$= \frac{N \times 20 \times 10}{4}$$

$$= N \times 50$$

Where 'N' is the number of cells counted.

Corrected WBC count: $\frac{\text{Uncorrected WBC } (10^9\text{Cells/L})*100}{\text{nRBC per 100 WBC}+100}$

9. Biological Reference

Adults: 4,000-11,000/cumm

At Birth: 10,000-25,000/cumm

1 to 3 years: 6,000-18,000/cumm

4 to 7 years: 6,000-15,000/cumm

8 to 12 years: 4,500-13,500/cumm

10. Critical Values

High Value	Low Value
>30,000/cumm	<2,000/cumm

11. Limitation

This process requires tidious mannual labour and also requires the performing technician/Medical lab technologist to have a good pipeting precision inorder to get good reproducible results.

12.Interference

As per manufacture instruction

13. Quality Control

Sample processed and reported the previous day can be used as QC to evaluate the results every morning or a third party QC sample can be analysed.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

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Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 19 of 21
Title: Differential Leukocyte Count		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for calculation of total leukocyte count in the whole blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

EDTA Anticoagulated Blood and Body fluids

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8^{o}C	Upto 72 hrs (3 days)
Whole blood	Room temperature	Upto 24hrs

5. Principle

Differential leucocytes counts are usually performed on blood films which are prepared on slides by the spread or wedge technique. The cells are evenly distributed on the slide as a monolayer. If there is clear fluids centrifuge and make smear.

The differential cell count expressed as a percentage of each type of cell. It is counted from standard area of smear (junction of body and tail) where RBC's touch each other.The polychromic staining solutions (Wright, Leishman) contain methylene blue and eosin. These basic and acidic dyes induce multiple colors when applied to cells. Methanol acts as fixative and also as a solvent. The fixative doesn't allow any further change in the cells and makes them adhere to the glass slide. The basic component of the white cells (i.e, cytoplasm) is stained by acidic dye and they are described as eosinophilic or, acidophilic. The acidic components (i.e, nucleus) take blue to purple shades by the basic dye and they are called basophilic.

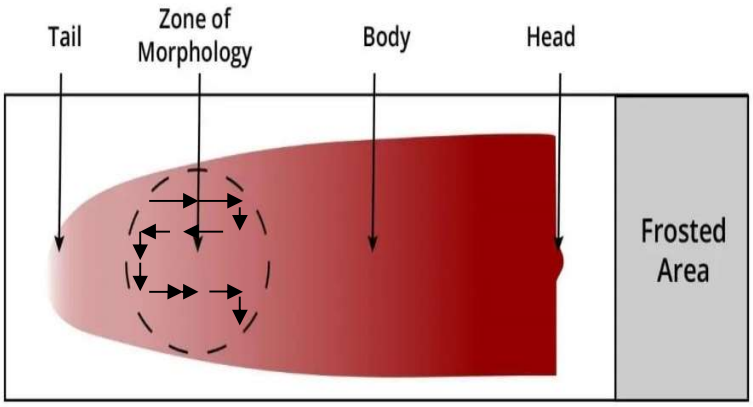
6. Materials and Reagents

- Differential blood cell counter
- stopwatch
- Microscope.
- Pipettes
- Wright stain
- Slide

7. Procedure:

1. Accept body fluids or whole blood, with properly filled work sheet.
2. Smear preparation:

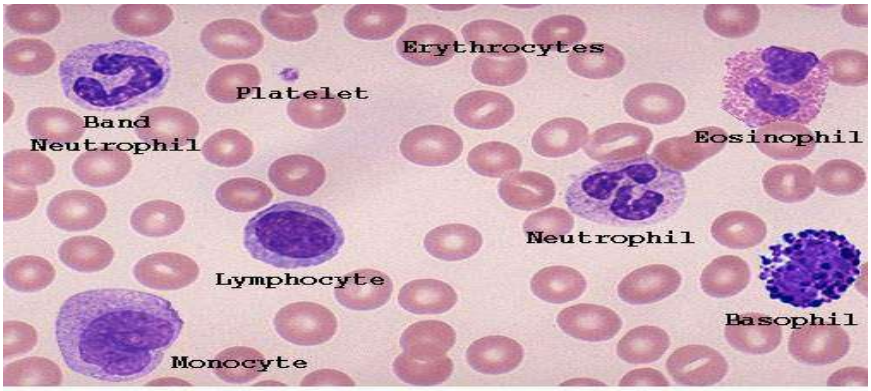
   Place one drop of mixed fluid or anticoagulated whole blood at one end of clean glass slide and make good smooth smear by using glass spreader.
3. Air dry.
4. Staining the slide as follows:
   a. Keep the slide on the staining rack and cover the smear with Wright stain for 3-5 minutes.
   b. Add equal volume of phosphate buffer (pH 6.8) over smear and blow gently on the surface.
   c. Wait for 10-15 minutes
   d. Wash thoroughly with tap water and air dry
5. Examination of smear:
   a. Place the slide on the microscope stage with the smear side up.
   b. Scan the blood smear using the X40 objective where there is no overlapping the cells.
   c. Place a drop of immersion oil on the smear and use 100x objective to focus it.
   d. Count the differential leucocytes up to 100 number as follows:



And give the reports in percentage on the work sheet.

8. Calculations

Blood element	% of leukocytes		Size (µ)	Cytoplasmic staining	Nucleus Morphology
Neutrophil	40-75		10-12	salmon-colored small granules	Segmented, 2-5 Lobes
Lymphocyte	20-45		7-8	Light blue, scant amount, no granules	Single large Oval purple
Monocyte	2-10		16-18	Basophilic, no granules	Large, kidney shaped
Eosinophil	1-6		13-14	Bright red coarse granules	Bilobed purplish
Basophil	0-1		14-15	Large, basophilic granules	Bilobed Bluish black



Wright-stained smear of normal blood (x1000) The RBC's are biconcave discs stained buff-pink, and the WBC's nucleus and cytoplasmic granules and platelet stain varying degrees of blue and pink.

9.0 Biological Reference

Neutrophil – 40-75%

Lymphocyte – 20-45%

Monocyte – 2-10%

Eosinophil – 1-6%

Basophil – 0-1%

10.0 Critical Values

Very high percentage of any one lineage of cell type must be properly reviewed. High percentage of cells looking like lymphocytes along with an increased number of total leucocyte count may be due to Leukemia.

11. Limitation:

The dilution buffer should be neutral buffer; otherwise it does not give good stain.

Note: please give the result in percentage of polymorph and monomorph only in case of Body fluid.

12. Interference

Slides used for smear preparation must be non-greasy and clean otherwise smears will have windows causing uneven distribution of cells.

13. Quality Control

Sample processed and reported the previous day can be used as QC to evaluate the results every morning or a third party QC sample can be analysed.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

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Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 22 of 25
Title: Erythrocyte Sedimentation Rate (ESR)		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for calculation of Erythrocyte Sedimentation Rate (ESR) in the whole blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

2ml fresh EDTA or 3.8% Citrate anticoagulated blood(4:1 ratio,4 part blood and 1 part anticoagulant).

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8°C	6hrs
Whole blood	Room temperature	Upto 2hrs

5. Principle:

The Erythrocyte Sedimentation Rate (ESR) is a relatively simple, non-specific test that has been used for many years to help detect inflammation associated with condition such as infection, cancers and auto immune diseases. The ESR expresses in mm per hour the rate at which red blood cells settle when anti coagulated blood is allowed to stand in a narrow tube (westerngren or wintrobe). It is shown by the height of the column of clear plasma at the end of one hour.

When anticoagulated blood is placed in a vertical glass tube & allows standing, undisturbed for period of time, the erythrocyte or RBCs will tend to fall towards the bottom, forming two layers, the lower layer is red cells & upper plasma. This process is called sedimentation. The length (mm) of fall of the top of the column of RBC in a given interval of time (1st hour) is the ESR.

Stage of ESR:

1. Stage of Aggregation
2. Stage of Sedimentation
3. Stage of packing

1. Stage of Aggregation:

It is the initial stage in which piling up of RBCs takes place. This phenomenon is known as Rouleaux formation. It occurs in first 10-15 minutes out of one hour.

2. **Stage of Sedimentation**

   It is the stage of actual falling of RBCs. In this stage, setting or sedimentation occurs at constant rate. This is related to both weight & surface area. Larger the aggregation form in stage first, the faster is the rate of falling. This occurs in 30-40 minutes out of 1 hour, depending upon the length of the tube used.

3. Stage of packing

This is the final stage & also knows as stationary phase. In this, there is slower rate of falling during which packing of the sediment RBCs in column occurs due to crowding. It occurs in final 10 minutes in 1 hour.

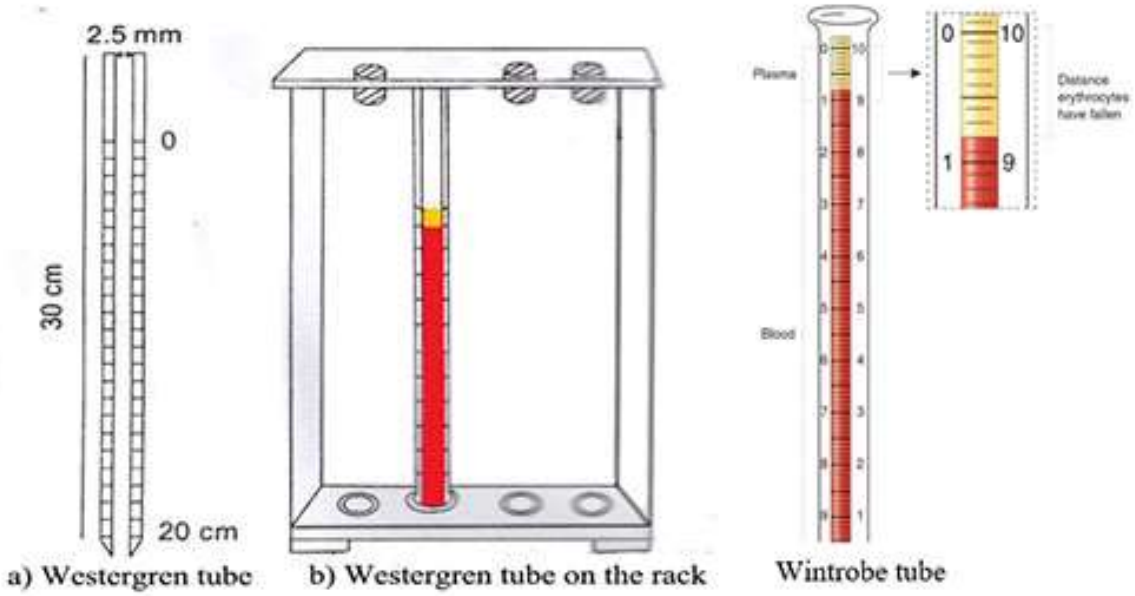
6. Reagents & materials:

- **For Westerngren Method**

1. Westergren rack
2. Westergren tube
3. Timer
4. 3.8 % trisodium citrated tube

7. Procedure:

- Accept properly filled work sheet and 2 ml citrated or EDTA blood sample. Incase EDTA blood sample, fill citrated tube up to the mark.
- Mix the blood properly and take out the cap of tube and insert the westergren ESR tube in to the blood tube, blood comes up to the 0 mark by capillary action.
- Make sure no air bubbles enter the tube.
- Place the ESR tube in the ESR stand.
- Immediately set timer for 1 hour.
- Exactly after 1 hr read how far the red cells layer has fallen. Give the result in mm per hour in work sheet.



B. Wintrobe's Method

Materials and Reagents

- EDTA Anticoagulated blood
- Pasteur pipette (syringe with long needle or ESR needle)
- Timer
- Wintrobe's stand
- Wintrobe's tube

Procedures:

1. Mix the anticoagulated blood thoroughly.
2. Fill the Wintrobe's tube by using Pasteur pipette up to **"0" mark**.
3. Place vertically in ESR stand & leave undisturbed for 1 hour.
4. At the end of 1 hour, read the result.

8. Calculations:

Record the result by reading the distance erythrocyte has fallen.

9. Biological Reference:

Westerngren's method

	0-50 yrs	51-85 yrs	>85 yrs
Male	<15 mm/ 1st hrs.	<20 mm/ 1st hrs.	<30 mm/ 1st hrs
female	<20 mm/ 1st hrs.	<30 mm/ 1st hrs.	<42 mm/ 1st hrs.

Wintrobe's Method

Male: 0-9 mm/ 1st hrs.
Female: 0-20 mm/ 1st hrs.

10.0 Critical Values

Very high values of ESR are seen in chronic diseases.

11. Limitation:

- ESR is not a diagnostic test but rather a prognostic test which is used to monitor treatment of chronic diseases.
- ESR is used as a screening method for all disease that is associated with a modification of the plasma proteins like globulin, albumin and fibrinogen.
- It is not a very reliable screening method as it can be raised where there is no disease and can be normal when disease is present.
- Is also does not indicates the type of disease.

12. Interference

Interferences which increase ESR:

- increased level of fibrinogen, gamma globulins.
- technical factors: tilted ESR tube, high room temperature.
- Chronic inflammatory disease(collagen and vascular disease increases ESR

Interferences which decrease ESR:

- abnormally shaped RBC (sickle cells, spherocytosis).
- technical factors: short ESR tubes, low room temperature, delay in test performance (>2 hours), clotted blood sample, excess anticoagulant, bubbles in tube.
- Polycythemia decreases ESR

13. Quality Control

Use of well calibrated ESR pipette, not letting formation of air bubble in ESR tube and noting the time exactly is mandatory for precise and accurate result.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

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https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01 Page No: Page 26 of 29
Title: Blood Grouping		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for determining the ABO and Rh group of human blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

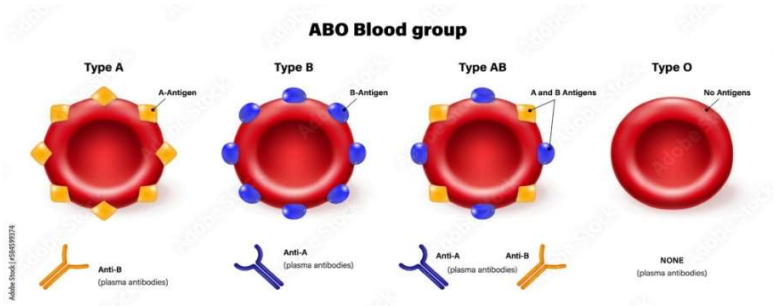
EDTA Anticoagulated Blood

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8˚C	Up to 72 hrs (3 days)
Whole blood	Room temperature	Up to 24hrs

5. Principle

ABO and Rh group are determined by detecting the presence or absence of A & B antigen in ABO system and presence or absence of D antigen in Rh system. The procedure used with antisera are based on the principal of agglutination. Normal human red cells possessing antigens, will clump in the presence of corresponding antibody.



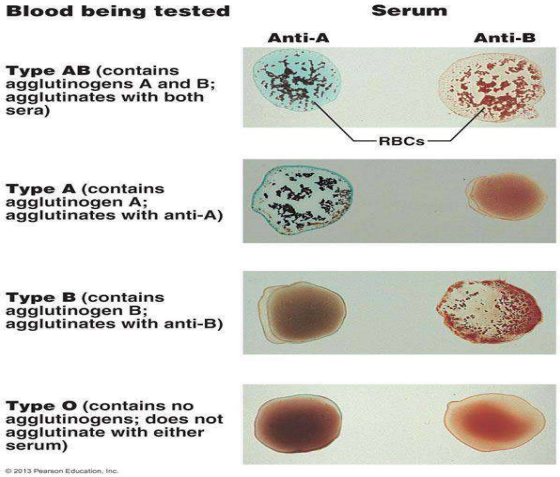
6. Materials and Reagents

1. Glass slides
2. Applicator sticks
3. Centrifuge
4. Test Tubes (10x75mm)
5. Microscope
6. Anti-A sera (blue colour): Human polyclonal or, murine monoclonal.
7. Anti-B sera (yellow colour): Human polyclonal or, murine monoclonal.
8. Anti-D sera(transparent): Human polyclonal or, human monoclonal

7. Test Procedures

A. ABO GROUPING BY SLIDE METHOD:

1. Bring the reagents at room temperature.
2. Mix blood tube gently.
3. Transfer one drop of whole blood for each test to a clean glass slide.
4. Transfer one drop of each reagent (antisera) to each blood drop.
5. Mix whole blood & antisera by using applicator.
6. Tilt the slide back and forth and observe for agglutination.



B. REVERSE GROUPING (SERUM GROUPING):

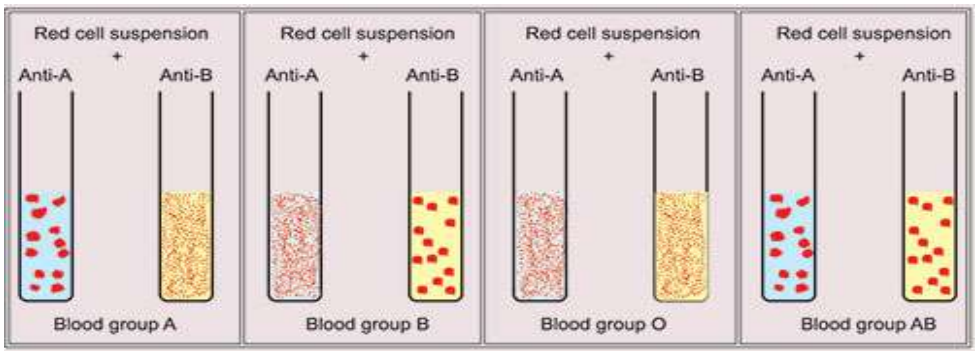
1. Label tubes with donor-patient and test identification.
2. Add 2 drops of test serum in all tubes in the corresponding column.
3. Prepare cells for testing of A, B and O groups by pooling 3 samples of each group.
4. Add 1 drop of 2% pooled A red cell suspension in tube labelled A_c.
5. Add 1 drop of 2% pooled B red cell suspension in tube labelled B_c.
6. Add 1 drop of 2% pooled O red cell suspension in tube labelled O_c
7. Mix the contents of the tubes gently and incubate the test for minimum 15 minutes at room temperature.
8. Centrifuge all tubes at 1000 rpm for 1 minute.
9. Gently resuspend the red cell button & examine for agglutination.

INTERPRETATION:

1. Agglutination in any tube of RBC tests and agglutination or haemolysis in serum test constitutes a positive test result. The expected agglutination reaction for positive tests are 3^+ to 4^+.
2. A smooth suspension of RBCs after resuspension of RBC button is a negative test result. All negative results must be verified under microscope. Cells should be separate without any clumping.
3. The interpretation of ABO group is as follows:

Serum Typing			Interception of ABO group		
Anti-A	**Anti-B**	**A_C**	**B_C**	**O_C**	
C	-	-	C/L	-	A
-	C	C/L	-	-	B
C	C	-	-	-	AB
-	-	C	C	-	O
C=Clumps, L=Lysis					

4. Resolve any discrepancies between cell and serum typing tests before the patient's or donor's ABO group is interpreted.



Note:

Confirm all Negative blood groups by D^u method as under

a. Wash the patient's cells at least 3 times with isotonic saline. After last wash decant supernatant saline completely.
b. Make 5-10% of cell suspension of the washed cells.
c. Add 1 or 2drops of AHG (Anti-Human Globulin)and a drop of anti-D to 100ul of washed cell suspension.
d. Centrifuge at 1000 rpm for1-2 minutes or 2000-3000 rpm for 20 sec to 1 min, gently resuspend the red cell button and read microscopically for agglutination.
e. A positive agglutination result indicates the presence of Rh0 variant (D^u) and must be reported as D^u positive or positive blood group.

8. Calculations:

Not Applicable

9. Biological Reference Range:

Not Applicable

10. Critical values

Not Applicable

11. Limitation

- False negative test may result from improper cell concentration, elevated incubation temperature and / improper centrifugation
- Presence of cold agglutinins may cause false positive test result.

12. Interference

Several clinical situations may result in unreliable serologic blood typing which can lead to mixed field reactions or discordances in the forward and reverse blood typing.

- **Recent blood Transfusions**:

Patients who receive type O transfusions in emergency situations will often develop a mixed field or discordant typing.

- **Elevated Globulin Levels**:

Patients with multiple myeloma, amyloidosis, hyperfibrinogenemia, Waldenstrom macroglobulinemia, plasma cell disorders or those who receive plasma expanders, such as dextran, may display a protein to plasma abnormality. This can lead to rouleaux formation and false appearance of agglutination on forward typing that may be inconsistent with reverse typing.

- **A_{weak}, B_{weak}, and Blood Type Subgroups**:

Antigen expression can become so weak that it is not detected by forward typing, with no natural antibodies present on the reverse reaction.

- **Age**:

Patients that are very young or elderly may have weakly reacting antibodies, or missing antibodies that renders the blood typing incongruent.

- **Immunosuppression**: Patients severely immunocompromised, due to disease, therapy, or depressed immunoglobulin levels may not mount an appropriate amount of antibody to reliably perform reverse typing, for the same physiologic reasons mentioned above.
- Serum from persons with agammaglobulinemia may not contain detectable ABO antibodies.
- As naturally occuring anti-A and anti-B are only formed 3-4 months after birth, it is not suitable for newborns and infants. Antibodies at this age are commonly of maternal origin.

13. Quality Control

If A and B group cells are available, reverse grouping should be performed to confirm the forward grouping.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

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Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 30 of 33
Title: Cross Matching		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory to check whether the donar and recepient blood are compatible or not i.e, whether there is any hemolysis or, agglutination when they are mixed together.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

i. Patient EDTA Anticoagulated Blood and Serum

ii. Donar's EDTA Anticoagulated Blood and Serum

4. Specimen Storage

Not Applicable

5. Principle

Cross matching is based on the principle of serological detection of any clinically significant irregular/unexpected antibodies in either donor or recipient's blood. There are two types of cross matches:

1. **Major Cross Match**: It involves testing the donor's red cells with recipient's serum to determine the presence of any antibody which may cause hemolysis or agglutination of donor red cells.This is more important than minor cross match.
2. **Minor Cross Match**: It involves testing of donor's plasma with recipient's red cells to determine the presence of any antibody which may cause hemolysis or agglutination of recipient's red cells.

6. Materials and Reagents

- Test Tubes
- Normal Saline
- Centrifuge

There are different methods for cross matching, as shown in table. Among them most commonly used technique is Anti-human globulin (AHG) cross match.

Method of Cross Match	Detects Antibody of Type :
Saline Cross Match	IgM
Albumin Cross Match	IgG
Anti-Human Globulin (AHG) Cross Match	IgG

7. Procedures:

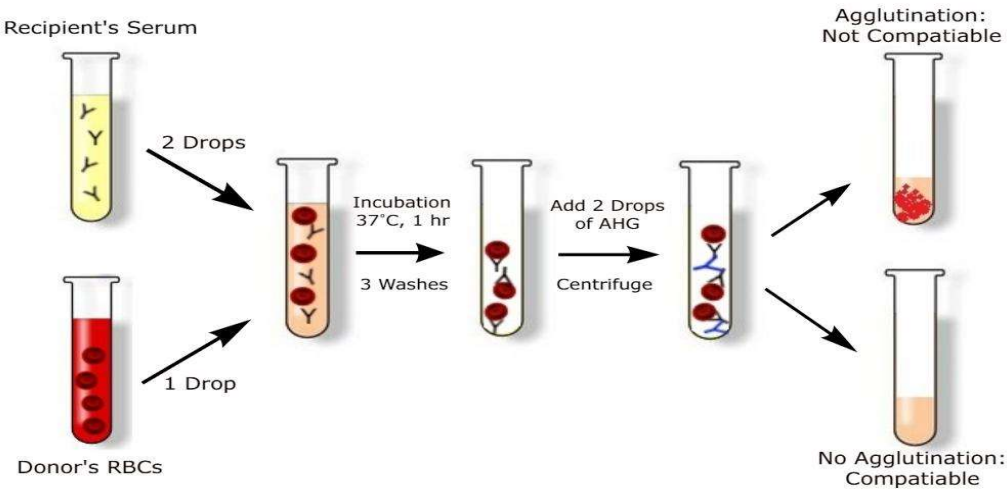
I. Saline Cross Match

1. Prepare 5% cell suspension of patient's blood (P) and donar's blood (D) in two separate tubes.
2. To the patient's tube, add two drops of patient's serum and one drop of donar cell suspension (major side).
3. To the Donar's tube (D), add two drops of donar's serum and one drop of patient's cell suspension (minor side).
4. Mix the contents of both the tubes gently and keep the tubes at room temperature for 30 minutes.
5. Centrifuge at 1,500 RPM for one minute.
6. Examine for the agglutinations both macroscopically and also microscopically.

II. Anti-Human Globulin (AHG) Cross Match

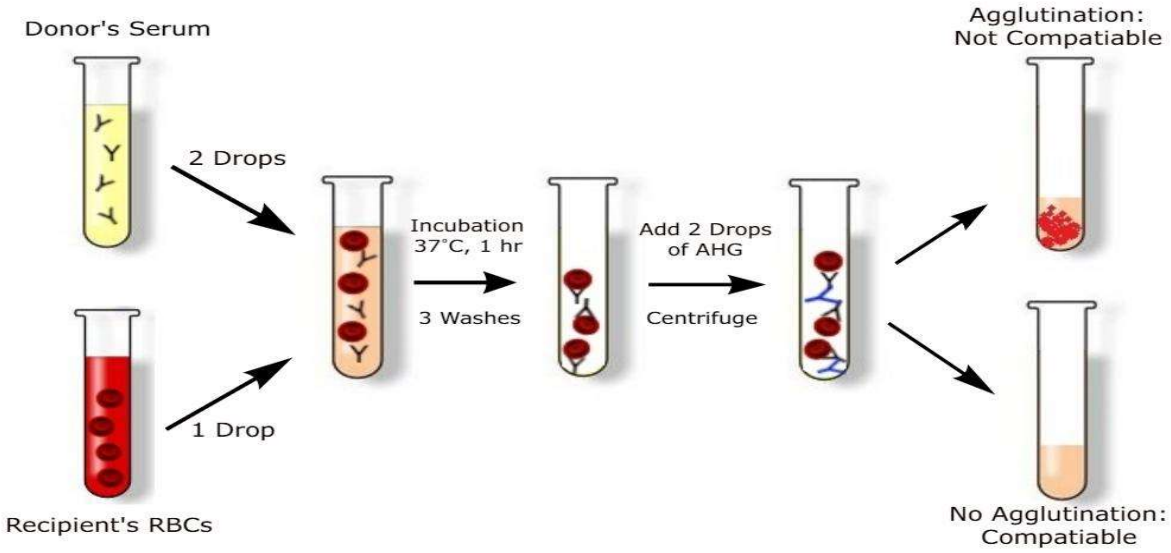
Major Cross Match

1. Prepare donor and recipient's blood sample: **Donor's red cells and recipient's serum/plasma**.
2. Prepare 3-5% saline cell suspension of red cells.
3. Label a test tube.
4. Add two drops of recipient's serum and one drop of donor cell suspension.
5. Mix and incubate the tubes at 37 degree Celsius for about 60 minutes.
6. Decant the serum completely and wash the cells three times in saline.
7. Add two drops of Anti-human Globulin (AHG) and mix. Allow to stand at room temperature for 5 minutes.
8. Centrifuge at 1500 rpm for 1 minute.
9. Observe macroscopically and microscopically for agglutination.
10. If macroscopic agglutination is not observed, transfer a small amount onto a glass slide and examine for microscopic agglutination. Rouleaux is not an indication of incompatibility.



Minor Cross Match

1. Prepare donor and recipient's blood sample: **Recipient's red cells and donor's serum/plasma**.
2. Label a test tube.
3. Add two drops of donor's serum and one drop of recipient's cell suspension.
4. Mix and incubate the tubes at 37 degree Celsius for about 60 minutes.
5. Decant the serum completely and wash the cells three times in saline.
6. Add two drops of Anti-human Globulin (AHG) and mix. Allow to stand at room temperature for 5 minutes.
7. Centrifuge at 1500 rpm for 1 minute.
8. Observe macroscopically and microscopically for agglutination.
9. If macroscopic agglutination is not observed, transfer a small amount onto a glass slide and examine for microscopic agglutination. Rouleaux is not an indication of incompatibility.



Interpretation

Compatible donor and recipient blood should show **no agglutination** in both major and minor cross match. Blood which shows incompatibility in major cross match should never be transfused, because the large plasma volume of the recipient blood containing antibodies can destroy the donor's red cells easily. The minor incompatibility is **less important** because the donor's serum which contains the antibodies is diluted in the recipient's own plasma, making the antibodies very dilute and ineffective.

8. Calculations:

Not applicable

9. Biological Reference Value:

Not Applicable

10. Critical Values:

Not applicable

11. Limitations:

12. Interference

13. Quality Control

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)
link -

https://drive.google.com/file/d/1h07QCTERDR0gnysjzbM2Ntu_zvCqL_hZ/view

https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01 Page No: Page 34 of 37
Title: Bleeding Time		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. **Scope and Application**:

   The Bleeding time (BT) is the only test to detect vascular defects and platelets disorder.

2. **Responsibility**:

   It is the responsibility of Laboratory personnel working in Hematology lab to carry out the test &report as required.

3. Specimen Type:

Not applicable

4. Specimen Storage

Not applicable

5. Principle:

The bleeding time test is used to evaluate how well a person's blood is clotting. It is the test that detects how long does it takes for blood to stop flowing from a standardized cut wound.

6. Materials Required:

- Sterile lancet or automated incision device
- Stopwatch or timer
- Filter paper or gauze pads
- Alcohol swabs
- Bandages
- Gloves
- Pen and recording sheet

7. Procedure:

A. IVY Method:

- A standard puncture (2-3mm depth) is made by a sterile lancet on the ear lobe and the time taken to stop the bleeding is recorded.

1. Clean the ear lobe with spirit swab.
2. Hold the glass slide behind the ear lobe for support.
3. Make a deep puncture(2-3mm)with a sterile lancet.
4. Start the stop watch and discard the glass slide.

5. Blot the drop of blood coming out from the incision every 30 second using a circular filter paper till the bleeding stops.
6. Note the time taken to stop the bleeding.
7. Report as required.

B. Estimation of Bleeding Time By Duke's Method

- Materials Required:

- Sphygmomanometer (blood pressure cuff)
- Sterile lancet or automated incision device
- Stopwatch or timer
- Filter paper or gauze pads
- Alcohol swabs
- Bandages
- Gloves
- Pen and recording sheet. Procedure

1. Procedure:

a. Preparation

- Explain the procedure to the patient and obtain informed consent.
- Ensure the patient is seated comfortably and relaxed.
- Wash hands thoroughly and wear gloves.

b. Site Selection

- Typically, the test is performed on the forearm, preferably on the volar surface.

c. Application of Blood Pressure Cuff

- Apply the sphygmomanometer cuff to the upper arm and inflate to 40 mmHg to standardize the capillary pressure.

d. Site Cleaning

- Clean the selected area on the forearm with an alcohol swab and allow it to air dry.

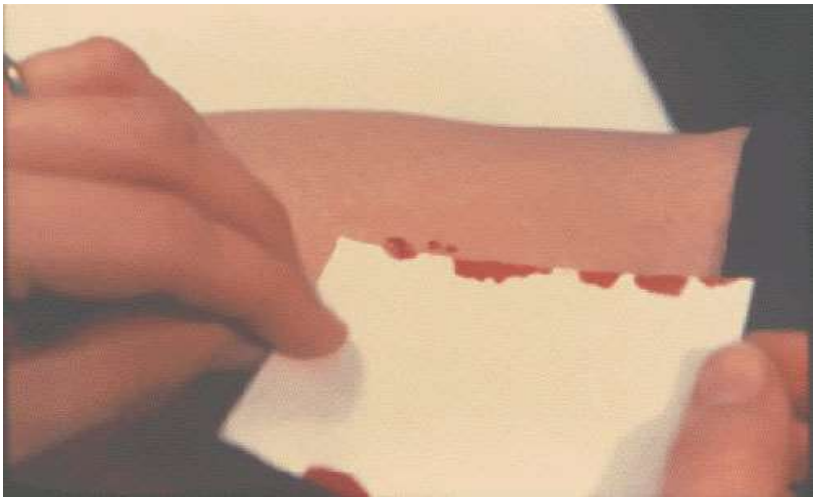
e. Making the Incision

Using a sterile lancet or an automated incision device, make a standardized incision on the forearm.

Start the timer immediately upon making the incision.

f. Blotting

- Every 30 seconds, gently blot the blood with filter paper or gauze pad. Avoid touching the wound directly with the filter paper to prevent clot disturbance.
- Continue blotting every 30 seconds until bleeding stops.



g. Recording the Time

- Stop the timer when bleeding stops completely.
- Record the bleeding time to the nearest second.

h. Post-Test Care

- Clean the incision site with a fresh alcohol swab.
- Apply a bandage to the incision site.
- Dispose of all used materials properly.

Interpretation of Results

- Normal bleeding time typically ranges from **2 to 9 minutes**.
- Results outside this range may indicate platelet function disorders or vascular abnormalities.

8. Calculations:

Not applicable

9. Biological reference range:

IVY method:

2-9 minutes

Duke's Method:

1-3 minutes

10. Critical values:

>15minutes

1. Platelets less than 50,000/cmm may lead to prolonged bleeding time.
2. A bleeding time of more than 10 minutes has a risk of bleeding; when it is 15 to 20 minutes, the bleeding risk may increase.

11. Limitations:

- Not a very reliable test

- The puncture wound may close before the cessation of bleeding.

12. Interference:

- Anemia prolongs BT
- Thrombocytopenia will have increased BT
- Aspirins, cefalothins, penicillin will prolong the BT
- Pediatrics and neonates require smaller incisions and pressure of 20mm of Hg

13. Quality Control

- Ensure all equipment is calibrated and functioning correctly.
- Regularly review and update the SOP to incorporate new guidelines or equipment.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)
link -

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https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%2 0Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 38 of 40
Title: Clotting time		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application:

Clotting Time is a mandatory test for screening of coagulation defects.

2. Responsibility:

It is the responsibility of a registered Laboratory personnel working in Haematology lab to carry out the test & report the test result.

3. Specimen Type:

Not applicable

4. Specimen Storage

The test must be performed as soon as the blood is collected, samples cannot be stored.

5. Principle:

Venous blood is collected in clean glass tube without any anticoagulant. The time taken for the blood to clot at 37 C is the clotting time (CT)

6. Reagents and Materials

- Sterile Disposable syringe and needle 20-22SWG
- Test tubes glass 12x75mm with rack
- Stopwatch or timer
- Water bath or incubator 37 ˚C
- Alcohol swabs
- Gloves
- Pen, Marker and recording sheet

7. Procedure

Method:

By Lee and White Method

a. Preparation

- Explain the procedure to the patient and obtain informed consent.
- Ensure the patient is seated comfortably and relaxed.
- Wash hands thoroughly and wear gloves.

Blood Collection

- Clean the selected venipuncture site with an alcohol swab and allow it to air dry.
- Using a sterile needle and syringe, draw a sufficient amount of blood from the patient.

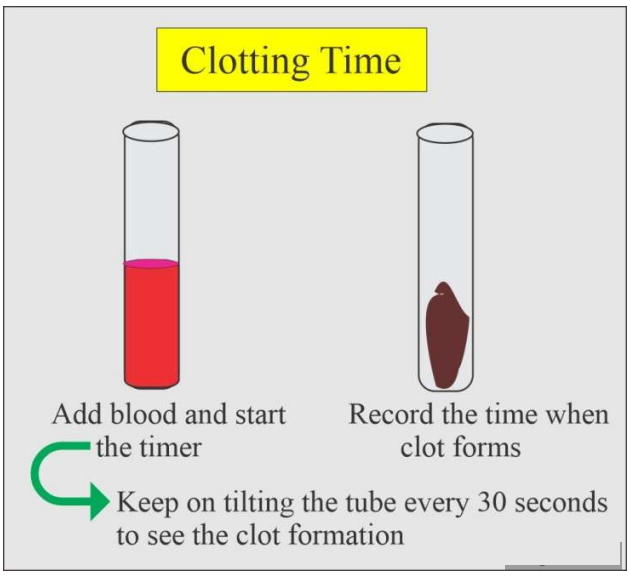
- Immediately transfer the blood into a clean, dry test tube.

c. Starting the Test

- Start the stopwatch as soon as the blood enters the test tube.

d. Observing Clotting

- Keep the test tube at room temperature or in a water bath/incubator at 37°C, as specified by the test method.
- Gently tilt the test tube every 30 seconds to observe the formation of a clot.



e. Recording the Time

- Stop the timer when a clot has formed and the blood no longer flows when the test tube is tilted.
- Record the clotting time to the nearest second.

f. Post-Test Care

- Dispose of all used materials properly.
- Apply a bandage to the venipuncture site if necessary.
- Ensure the patient is comfortable and monitor for any immediate adverse reactions.

g. Interpretation of Results

- Normal clotting time typically ranges from 5 to 15 minutes, depending on the method used.
- Results outside this range may indicate coagulation disorders or abnormalities in the intrinsic pathway.

8. Calculations:

Note the time for blood to clot in both the tubes and give result as average of them.

9. Biological reference Value:

6-15 minutes

10. Critical values:

> 30 minutes

11. Limitations:

1. This test is insensitive, so it lost its value.
2. There are many variables in the technique of performing the test.
3. This fails to detect the moderate deficiency of coagulation factors.
4. This test is only prolonged in severe deficiency.
5. Normal clotting time is despite prolonged bleeding time seen in thrombocytopenia.
6. This may be normal in patients taking anticoagulant therapy.
7. This is usually normal when the intrinsic and common pathways are present in an amount not exceeding 1% of the normal plasma level.

12. Interference:

- Poor venipuncture technique resulting in hemolysis alters the clotting time
- Bubbles in syringe increases the clotting time
- Dirty tubes will affect the result
- Diameter of the tube alters the result, smaller the diameter less is the clotting time

13. Quality Control

- Ensure all equipment is calibrated and functioning correctly.
- Regularly review and update the SOP to incorporate new guidelines or equipment.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)
link -

https://drive.google.com/file/d/1h07QCTERDR0gnysjzbM2Ntu_zvCqL_hZ/view

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Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01 Page No: Page 41 of 43
Title: Prothrombin Time (PT)		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application:

Prothrombin Time is a mandatory test for screening of coagulation defects.

2. Responsibility:

It is the responsibility of a registered Laboratory personnel working in Haematology lab to carry out the test & report the test result.

3. Specimen Type:

- Obtain venous blood by clean vein puncture. Immediately mix 9 part of blood with 1 part 3.2% buffered sodium citrate as anticoagulant. Avoid foaming the specimen.
- Centrifuge blood for 15 minutes at 2000 rpm. Specimen maintained at 22 - 24˚C must be tested within 2 hours, the separated plasma sample can be preserved frozen at -20˚C up to 2 weeks or at -70˚C up to 6 months.

4. Specimen Storage

The test must be performed as soon as the blood is collected , samples cannot be stored for more than 2hrs of collection at room temperature.

Specimen Type	Storage Temperature	Time Interval
Whole blood	22-24˚C	Upto 2 hrs
Citrated Plasma	-20˚C	Upto 2 weeks
Citrated Plasma	-70˚C	Upto 6 Months

5. Principle:

Tissue thromboplastin is a highly sensitive reagent that is used to perform the one stage prothombin Time (PT). Prolongation of PT indicates either acquired or congenital disorder that effect coagulation factor I, II, V and X. The PT has been widely accepted as the means to monitor, patients on oral anticoagulant therapy due to the reduction in the activity of vitamin K dependent clotting factor(II, VII, IX, X, protein C & S). Thromboplastin can be used to assay coagulation factors in the extrinsic and common pathway of coagulation.

The one stage Prothombin Time (PT) measures the clotting time of plasma after the addition of optimal amount of thromboplastin and calcium. The time to formation of a fibrin clot is then measured.

6. Reagents and Materials:

- Thromboplastin reagent, lyophilized
- Calcium chloride

a. Reagent Preparation:

Reconstitute 1 vial of thromboplastin reagent with the entire content of one vial of buffer or some time supply the reagent ready to use. Mix reagent by gentle inversion prior to use.

7. Procedures:

- Accept 2 ml of citrated blood sample with properly filled work sheet.

a) Manual Method:

- Thoroughly mix the blood without foaming.
- Centrifuge at 2000 rpm for 15 minutes and remove the plasma and keep at 37^0C.
- Pipette 200µl thromboplastin reagent into a test tube and warm at 37^0C for 3 minutes.
- Add 100µl pre warmed plasma into the tube by quickly blowing out contents of the pipettes and starting the stop watch simultaneously.
- Immediately mix the contents of the tube by gentle shaking it in the water bath.
- After 8-9 seconds remove the tube and examine for clot formation by gently by quick tilting the tube, stop the watch at the first sign of a clot
- Run normal and abnormal control sera simultaneously.

b) Coagulotmetric method

Set the programme to PT

- Incubate required amount of plasma in a cuvette inside the inbuilt incubator of the coagulometer.
- Put 200 µl of thromboplastin reagent into the cuvette and warm it for 3 minutes.
- After 3 minutes, pipette 100 µl of pre warmed plasma and look at the screen for the PT result in seconds along with INR.

For any kind of trouble shooting, follow the user manual of Coagulometer.

8. Calculation:

The result can be reported in seconds. The international committee for standardization in hematology and the international committee on thrombosis and homeostasis have agreed on recommendation for the reporting of PT result as an International Normalized Ration (INR). The INR is based on the international sensitivity index thromboplastin reagents, PT reagents are assigned as ISI value by calibration (IRP) with an assigned ISI of 1.0. The INR is calculated using the following formula:

$$INR = \left[\frac{\text{PT Patient}}{\text{PT Reference Plasma}}\right]^{ISI}$$

ISI: Lot specific international sensitivity index for the reagent / instrument system

- The PT may be falsely prolonged by substances like corticosteroids, EDTA, oral contraceptives, asparaginase , chlofibrate erythromycin, ethanol, tetracycline and anti coagulants such as heparin and warfarin.
- The PT may be shorten by substances including anti histamine, caffeine, oral contraceptives, phenobarbital and vitamin K.

9. Biological Reference values:

10– 14 Seconds

10. Critical values:

>37 seconds

INR>5.0

11. Limitations:

- A large number of drugs can interfere with the action of warfarin in vivo, either potentiating or inhibiting its effect on the coagulation factors II, VII, IX, and X.
- The Prothrombin Time is insensitive to unfractionated heparin up to approximately 2.0 units per mL.
- Inhibitors such as the lupus anticoagulant may interfere with Prothrombin Times.
- Direct thrombin inhibitors (Argatroban, Bivalarudin, etc) in therapeutic doses will result in prolonged Prothrombin Times.
- Patients with abnormally elevated hematocrits may show falsely prolonged PT.

12. Interference:

- Clot in sample may result in falsely elevated PT values
- Patients under medication may have High PT values
- Improper blood-to-anticoagulant ratio.
- Contamination with saline, heparin, or other anticoagulants

13. Quality Control:

- Reliability of test result should be monitored by use of normal and abnormal control plasma.
- In case, the value of control plasma is out of the given ranges, check the calibration, reagent, its stability and the performance of the analyzer.
- COMMERCIAL CONTROLS – are available that will cover the clinical significant range. Prepare reagents as per the manufacturer's instructions. The value of normal control should range between 10 and 14 seconds.
- MNPT (mean Prothrombin Time) – this value is the mean value of the pooled plasma done on each new lot number of PT reagent. MNPT values are used to calculate the INR.
- **Calibration**:

  Coagulometer should be frequently checked and calibrated for its precision and accuracy.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

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Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 44 of 45
Title: Activated Partial Thromboplastin Time (APTT)		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for determining the Activated Partial Thromboplastin Time in human blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

- Obtain venous bloods by clean vein puncture, immediately mix 9 part blood with 1 part 3.2% buffered sodium citrate as anticoagulant. Avoid foaming the specimen.
- Use only plastic or silicone glass containers.
- Centrifuge blood for 15 minutes at 1500g, cover specimens to prevent pH changes that may affect test results.
- Turbid; Icteric; Lipemic and hemolysed specimen are not assayed.
- Specimen maintained at 22 - 24°C must be tested within 2 hours, the separated plasma sample can be frozen at -20°C up to 2 weeks or at -70°C up to 6 months.

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	22-24°C	Upto 2 hrs
Citrated Plasma	-20°C	Upto 2 weeks
Citrated Plasma	-70°C	Upto 6 Months

5. Principle:

The Activated Partial Thromboplastin Time (APTT) is a simple and versatile test which is sensitive to deficiencies of all plasma clotting factors except Factor VII. However it is mainly used to detect deficiencies in Factor VIII, IX, XI, XII and Prekallikrein (Fletcher Factor).

Activated partial thromboplastin time is performed by adding an aPTT reagent containing a plasma activator and phospholipid to the test specimen, the phospholipid serves as a substitute for platelet. The mixture is incubated for activation, then re calcified with calcium chloride and clot formation is timed.

6. Reagent and Materials:

- APTT reagent (Rabbit brain phospholipids -0.007%, ellagic Acid- 0.0037 %)
- Buffer, Salts and Stabilizers
- Calcium chloride (Cacl2) -0.02 mol/l
- **Storage and Stability:**

Store at 2-8°C. Do not freeze. Open vials are stable for 30 days when store at 2-8°C

7. Procedure:

A. Manual Method :

- Accept 2 ml of citrated blood sample with properly filled worksheet
- Pipette 100µl plasma into test tube.
- Add 100µl APTT reagent, mix and leave the tube for 5 minutes at 37°C.
- Add 100µl calcium chloride (pre warmed) solution into the test tube and start the stop watch immediately.
- Mix and leave undisturbed for 20 seconds.
- After 20 seconds lift up and tilt the tube gently to observe the clot.
- Immediately stop the stopwatch and note at first sign of clot.

B. Automated Coagulometer Method:

- Strictly follow the Instrument procedure manual

9. Biological Reference values:

29– 31 Seconds

10. Critical values:

>100 seconds

11. Limitations:

Oral contraceptives, estrogen, pregnancy, Coumadin type drugs, heparin, asparaginase and naloxone has been reported to influence the APTT test.

12. Intereference:

- Blood specimens from patients treated with lytic agents usually contain heparin that adds to the prolongation of the PTT.
- Patients with abnormally elevated hematocrits may show falsely prolonged PTT.

13. Quality Control:

Routine quality control is indispensable in coagulation testing. Abnormal and normal control plasma should be tested in conjunction with patient's plasma.

14. Waste Disposal

- As per Healthcare Waste Management guidelines (Government Guideline)

link -

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https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01 Page No: Page 46 of 48
Title: Platelets Count		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for calculation of red blood cell in the human blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

EDTA Anticoagulated Blood

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8°C	Up to 72 hrs (3 days)
Whole blood	Room temperature	Up to 24hrs

5. Principle

The Blood specimen is diluted 1:200 with the RBC diluting fluid and the cells are counted under high power (40X objective) by using a counting chamber. The number of cells in undiluted blood are calculated and reported as the number of red cells per cumm of whole blood.

6. Materials and Reagents

- Pipette
- Test tubes
- Test tube rack
- Improved Neubauer Counting Chamber (hemocytometer)
- Counting chamber cover glass
- Microscope.
- Hand counter
- RBC diluting fluid

Compositions:

Mercuric chloride	0.25gm
Sodium sulphate	2.50gm
Sodium chloride	0.50 gm
Distilled water	100.0 ml

Final pH (at 25°C) 5.9±0.1

7. Procedures

- **Micro Dilution method:**

1. Measure 3980 µl of diluting fluid & dispense it into a tube.
2. Add 20 µl of well-mixed EDTA anticoagulated venous blood.

3. After adequate mixing, counting chamber is charged.
4. Leave the chamber undisturbed for 2 min. to allow time for the white cells to settle.
5. After 2 min. RBCs are counted in 5 squares of the central square.
6. Then using the formula, total number of RBCs are counted.

8. Calculation:

- Number of cells counted = N
- Counted area = (A) = 5/25 i.e. 1/5
- Chamber depth = (d) = 1/10 mm
  i.e. depth factor = 10
- Dilution = 1:200
- Dilution factor =200

RBCs count Formula:

Calculation :

$$\text{RBCs} = \frac{\text{Number of RBCs/cu.mm of whole blood}}{\text{Number of RBC Counted x Dilution}} = \frac{\text{Number of RBC Counted x Dilution}}{\text{Area Counted x Depth of the Fluid}}$$

Here,

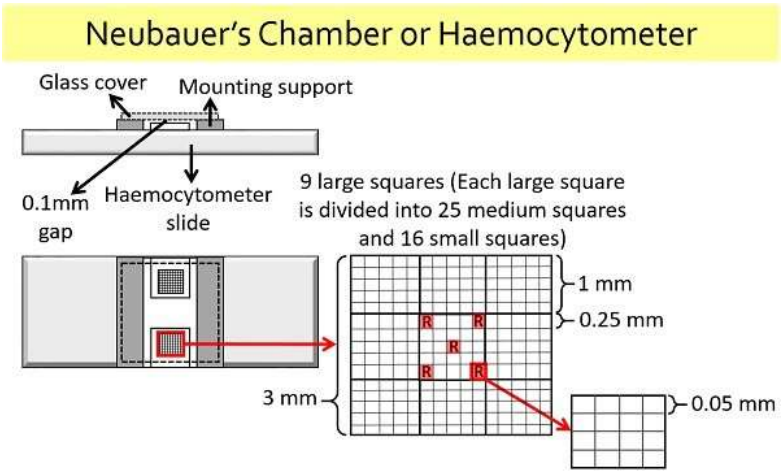
Dilution = 20

Area Counted = 5 x 0.04 Sq.mm = 0.2 Sq.mm

Depth of the fluid = 0.1 mm (Constant)

Hence,

$$\text{Number of RBCs/cu.mm of whole blood} = \frac{\text{Number of RBC Counted x 200}}{0.2\,\text{Sq.mm x0.01 mm}} = \text{Number of RBC Counted x 10000}$$



9. Biological Reference:

In Males – 4.8-5.5 million/mm^3

In Females – 3.5-5 million/mm^3

10. Critical Values:

Not applicable

11. Limitation

The movement of cells in the chamber during the filling process causes then to collide and this influences their distribution.

12. Interference

Take care while drawing samples for RBC count, hemolysed samples cannot be analysed for this test.

13. Quality Control

Use of well calibrated pipette is mandatory for precise and accurate result

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

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Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 49 of 50
Title: Red Blood Cells Count (RBC Count)		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for calculation of Platelets.

2 Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

EDTA Anticoagulated Blood

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8˚C	6hrs
Whole blood	Room temperature	Up to 2 hrs

5. Principle:

The anticoagulated blood is diluted with platelets diluting fluid which is hypotonic to RBCs but isotonic to platelets. It is slightly alkaline hence dissolves cell stroma & allows platelets to be counted under high power of microscope in diluted blood & reported as total platelets per cubic mm of blood.

6. Materials and Reagents

- Pipette
- Test tubes
- Test tube rack
- Improved Neubauer Counting Chamber (hemocytometer)
- Counting chamber cover glass
- Microscope.
- Hand counter
- Platelet diluting fluid

Composition:

Ammonium oxalate Powder	1gm
Distilled water	100ml

7. Procedure:

Bulk dilution method

1. Take 50µl well mixed EDTA blood + 950 µl of 1% ammonium oxalate in a clean & dry test tube.
2. Incubate 10 minutes at 37^0C for complete lysis of RBCs.
3. Charge the counting chamber & place inside the petri dish with moist filter paper & leave it for 15 minutes (settlements time). This prevent drying & allows enough time for settlement of platelets.
4. Focus under low power & then count under high power.
5. Platelet appears highly retractile.
6. Count the platelets where RBCs are counted.

8. Calculations:

$$Total\ platelets\ count\ per\ cumm = \frac{N\ x\ dilution\ factor\ x\ depth\ factor}{Counted\ area}$$

$$= \frac{N\ x\ 20\ x\ 10}{1/5}$$

$$= \frac{N \times 200 \times 10}{1/5}$$

$$= N \times 1000$$

Where 'N' is the number of cells counted

9. Biological Reference

1,50,000 – 4,00,000/cumm of blood

10. Critical Values

	High Value	Low Value
For Adult	>10,00,000/cumm	<50,000/cumm
For Pediatric	>1,00,000/cumm	<20,000/cumm

11. Limitation

Delay on platelet count i.e, more than 2 hours after blood collection causes clumping and disintegration of platelets.

12. Interference

Platelet count may be falsely decreased in sample improperly drawn.

13. Quality Control

Commercially available QC samples can be used to access the precision and accuracy of the test.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

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https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 51 of 53
Title: Packed Cell Volume		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

- The test will be used in the clinical laboratory to measure the packed cell volume i.e, the proportion of blood occupied by red blood cells, usually expressed as a percentage of the total blood volume.

2 Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3 Specimen Type

EDTA Anticoagulated Blood

4. Specimen Storage

Not Applicable

5. Principle

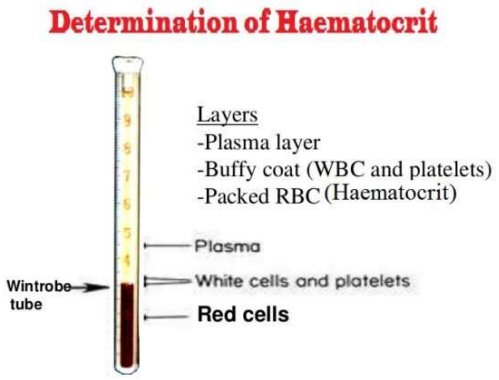
Blood is collected by vein puncture, mixed with EDTA & introduced into wintrobe's tube. The tube is then centrifuged to pack the cellular elements. The height of the pack red cells column is read & reported as percentage of the total blood volume. This percentage value is the PCV.

6. Materials and Reagents

- Pasteur pipette (syringe with long needle of ESR needle)
- Centrifuge machine
- Wintrobe's stand

7. Procedures:

1. Fill the hematocrit tube to 10 mark with well mixed EDTA blood with the help of a long Pasteur pipette. The blood column must be free from air bubbles.
2. Place the tube in centrifuge & spin at 3000 RPM for 30 minutes.
3. Read the upper level of the column of RBCs i.e. volume occupied by RBCs after packing, expressed as percentage of the total volume of blood.



8. Biological Reference

Male: 40% - 54%
Female: 36% - 47%
At birth: 44% - 62%
1 year: 35%
10 years: 34% - 41%

9. Critical Values

High Value	Low Value
>60%	<20%

Note the following observations: The following observation of plasma color and Buffy coat Volume may give following indications

1. **Color :**

Colour & opacity of plasma	Expected condition
Yellow	May be jaundice
Milky	Lipemia
Cloudy	May be multiple myeloma
Reddish	Haemolysis

1. Buffy layer :

Normally it is 0.5 – 1.0 mm. Each 0.1 mm = 1000 cells/cu.mm (approximately).

10. Limitations

PCV should be determined within 6 hours after the blood collection.

11. Interference:

Air bubble formation while filling the WSintrobe's tube may give erroneous PCV value.

13. Quality Control

Use of well calibrated Wintrobe's tube, not letting formation of air bubble in the tube and a well calibrated centrifuge mandatory for precise and accurate result.

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

https://drive.google.com/file/d/1h07QCTERDR0gnysjzbM2Ntu_zvCqL_hZ/view

https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01 Page No: Page 54 of 55
Title: Red Cell Indices		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in clinical laboratory to calculate red cell indices after calculating Hb, PCV & RBCs counts.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

EDTA Anticoagulated Blood

4. Specimen Storage

Not Applicable

5. Principle

Red cell indices provide quantitative information about RBCs & are guides to the classification of anemia.

6. Materials and Reagents

Not applicable

7. Procedures:

1. **MCV (Mean Cell Volume)**

   The mean cell volume is the mean volume of the red cells or the volume of the average red cells expressed in femto liters (fl). 1fl = 10^{-15} liters

$$\mathbf{MCV} = \frac{\mathbf{PCV}}{\mathbf{RBCs\ IN\ Millions}} \times \mathbf{10}$$

2. **MCH (Mean Cell Hemoglobin)**

   It is the amount of haemoglobin present in the average RBCs. It is expressed in pico gram (pg).

   1pg = 10^{-12} gm

$$\mathbf{MCH} = \frac{\mathbf{Hb(gm\%)}}{\mathbf{RBCs\ in\ millions}} \times \mathbf{10}$$

3. **MCHC (Mean Corpuscular Hemoglobin Concentration)**

   The amount of hemoglobin in 100 ml of packed red cells or the concentration in the average red cell. It is expressed in gm%.

$$\text{MCHC} = \frac{\text{Hb}}{\text{PCV}} \times 100$$

8. Calculations:

As per above

9. Biological References

MCV: 87±5 fl

MCH: 27-32 pg

MCHC: 32-36 gm/dl

10. Limitations

Generally high percentage of error occurs due to error in erythrocyte count which is overcome in the automated coulter counter.

11. Interference:

Error in RBC count and PCV value gives wrong RBC indices values

12. Quality Control

Not applicable

13. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

https://drive.google.com/file/d/1h07QCTERDR0gnysjzbM2Ntu_zvCqL_hZ/view

https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01 Page No: Page 56 of 57
Title: Absolute Eosinophil Count		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for calculation of Absolute Eosinophil Count in the human blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

EDTA Anticoagulated Blood

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8°C	6hrs
Whole blood	Room temperature	Upto 2 hrs

5. Principle

Eosin 'Y' stains the eosinophil, formalin fixes the cell & phenol prevents the eosinophil from the lytic action of water.

6. Materials and Reagents

- Microscope.
- Counting Chamber.
- Eosinophil diluting fluid: - also called **Hingleman's Fluid**.

Composition:

- Eosin Y – 0.5g (stain the cell)
- 95% phenol – 0.5ml (prevent the lysis of eosinophils)
- Formalin – 0.5ml (fix the cells)

D/W – up to 100 ml (lytic action**)**

7. Test Procedures

1. Take 20µl of well mixed EDTA blood & dilute with 180 µl of diluting fluid in a test tube.
2. Dilution should be 1 in 10.
3. Mix it & keep for 10 minutes before charging the counting chamber.
4. Immediately, charge in the counting chamber after mixing well to prevent clumpimg of eosinophils.
5. Keep loaded chamber in a moist Petri dish for 15 minutes in order to lyse the RBCs & prevent evaporation.

6. Focus the ruled area under low power objective & count the number of eosinophils in those area (squares) where total WBCs are counted.

8. Calculation:

Number of cells counted = N
Area counted = 4 mm^2
Dilution factor = 10
Depth factor = 10

$$Absolute\ Eosinophil\ count/cumm = \frac{N\ \times depth\ factor\ \times dilution\ factor}{Area\ Counted}$$

$$= N \times 10 \times 10/4$$
$$= N \times 25$$

OR, to estimate the total eosinophil count (AEC), first find out complete blood count (CBC). The values of white blood count (WBC) and eosinophil percentage (EOS%) are then use this formula that finds the Absolute Eosinophil Count:

$$\%\ Eosinophils = \frac{Absolute\ count\ x\ 100}{Total\ leukocyte\ count}$$

9. Biological Reference:

40 - 440/cumm

10. Critical Values:

Not applicable

11. Limitation

Eosinophils disintegrate in the diluting fluid hence the count should not be delayed for more than 30 minutes after diluting the blood.

12. Interference

As per manufacture instruction.

13.Quality Control

Not applicable

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

https://drive.google.com/file/d/1h07QCTERDR0gnysjzbM2Ntu_zvCqL_hZ/view
https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 58 of 60
Title: Reticulocyte Count		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for calculation of Reticulocyte Count in the human blood.

2. Responsibility

It is the responsibility of the registered laboratory personnel to perform the test.

3. Specimen Type

EDTA Anticoagulated Blood .

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8°C	6hrs
Whole blood	Room temperature	Upto 2 hrs

5. Principle

Reticulocytes are juvenile red cells. They contain remnants of the ribosome's and the ribonucleic acid which are present in large amounts in the cytoplasm.The RNA in the polychromatic erythrocytes is precipitated by staining them while they are still alive. This appears as a dark blue network or reticulum in some of the cells (reticulocytes).

6. Reagent and Material:

- Dissolve 1.0 gm of brilliant cresyl blue or new methylene blue or azure B thicohate in 100 ml of osmotic phosphate buffer of pH 7.4.
- NaH2PO4.2H2O(150 mmol/l) – 23.4 gle – 18ml)
- Na2HPO4 (150 mmol/l) – 21.3 gle – 82ml
- Test tube stand / Glass Slide / Spreader
- Water bath

7. Procedure:

1. Accept 2 ml EDTA blood sample with properly filled worksheet.
2. Delivered 2-3 drops of stain in to a test tube and add equal volume of well mixed blood sample.
3. Mix the tube gently and leave in water bath.
4. At end of this time resuspend the red cells by gentle mixing and make a smear in the usual way and air dry.
5. Choose an area of the field where the cells are undistorted and the staining is good.
6. Using x100 objective count the number of reticulocytes seen per 100 of RBC.

7. Give the report in percentage on the work sheet.

8. Calculation

Reticulocyte appears as cell containing dark blue granules or blue network.

Count 1000 RBCs including reticulocytes, the number of reticulocyte is reported as percentage of the total RBCs.

$$\text{Reticulocyte count (\%)} = \frac{\text{Number of reticulocyte counted x 100}}{\text{Number of RBC examined}}$$

$$\text{Absolute count of reticulocyte / cu.mm of blood} = \frac{\text{Reticulocyte count (\%) x RBC count / cu.mm}}{100}$$

NOTE:CORRECTED RETICULOCYTE COUNT

- In states of anemia, the reticulocyte percentage is not a true reflection of reticulocyte production. A correction factor must be used so as not to overestimate marrow production, because each reticulocyte is released into whole blood containing few RBCs - a low hematocrit (Hct) - thus relatively increasing the percentage.
- The corrected reticulocyte count my be calculated by the following formula:

$$\text{Corrected reticulocyte count} = \text{Retic \% x} \frac{\text{patient's Hct \%}}{\text{Average normal hct*}}$$

*Average normal hct is 45 for men and 42 for women.

9. Biological Reference Value:

- Adults & Children : 0.5 – 2.5 %
- Infants: 2.0 – 6.0

According to age:

Age	%
Newborn	3 to 7
0ne day	3 to 7
3 days	1 to 3
7 days	0 to 1
one month	0.2 to 2.0
1.5 month	0.3 to 3.5
2 month	0.4 to 4.8
2.5 month	0.3 to 4.2
3 month	0.3 to 3.6
4 to 12 month	0.2 to 2.8
Adult	0.5 to 1.5

According to Milestone:

- Adult/elder/child = 0.5 to 2%
- Infants = 0.5 to 3.1%
- Newborn = 2.5 to 6.5%
    - Reticulocyte index = 1.0

10. Critical Values:

Not applicable

11. Limitation

1. It is inferior to new technology like flow cytometry.
2. It is not very accurate method of reticulocyte so it is better to report the result as approximate percentage.

12. Interference

As per manufacture instruction.

13. Quality Control

Not applicable

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

https://drive.google.com/file/d/1h07QCTERDR0gnysjzbM2Ntu_zvCqL_hZ/view

https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 61 of 62
Title: Peripheral Blood Smears Examination		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Scope and Application

The test will be used in the clinical laboratory for examination of peripheral blood smears to study the various blood cells and hemoparasites.

2. Responsibility

It is the responsibility of the registered laboratory personal to perform the test.

3. Specimen Type

EDTA Anticoagulated Blood .

4. Specimen Storage

Specimen Type	Storage Temperature	Time Interval
Whole blood	2-8°C	6hrs
Whole blood	Room temperature	Up to 2 hrs

5. Principle

A blood smear is a laboratory procedure for examination of a small drop of blood spread over a glass slide. This blood test provides information about the number, quality and shape of all blood cells by visual inspection by microscope.

A drop of blood is placed on one end of a slide. It is then spread into a thin film while holding a spreader at a 45° angle.

6. Material and Reagents:

a. Clean grease free glass slides

b. Spreader slide

c. Diamond pen or Pencil

d. Pipette

e. Wright stain solution

7. PROCEDURES

- Take clean, dry glass slide.
- Place small drop of blood at the center of the quarter edge of one end of the slide.
- Immediately, place the spreader at an angle of 45° to the slide and move it back to touch the drop of blood.
- The drop spread out quickly along the line of contact of the spreader with the slide.
- Then spread the film with a rapid, smooth, forward movement of the spreader.
- Air dry and stain with Wright's stain.

- After completion of staining procedure, air dry the smear and examine the smear by using 10X, 40X and finally at oil immersion objective, where the RBC's are just apart from each other.

8. RESULTS AND INTERPRETATION

Morphologic Reports:

a. Red blood cells:
   i. Color: Normochromic, Hypochromic, Hyperchromic.
   ii. Size: Variation of size of RBC like Normocytic, Macrocytic, Microcytic (Anisocytosis)
   iii. Shape: Variation on shape of RBC like sickle cells, target cells, spherocytes, ovalocytes, tear shape, pencil shape (Poikilocytosis).

b. Blood parasites : Sporozoa, Nematodes, Trypanosomes, Spirochetes etc

c. White Blood Cells: Immature cells, toxic granulations and numbers.

d. Platelets: Numbers and morphology.

9. Biological Reference Value

Not applicable

10. Critical Values

Not applicable

11. LIMITATIONS

- Poorly made smear
- Dirty/greasy glass wares
- Incorrect buffer pH
- Unfiltered stain

12. Interference

- Windows in the slides make smear study difficult.
- Improper staining and buffer PH may alter the result quality

13. Quality Control

Not applicable

14. Waste Disposal

As per Healthcare Waste Management guidelines (Government Guideline)

link -

https://drive.google.com/file/d/1h07QCTERDR0gnysjzbM2Ntu_zvCqL_hZ/view
https://climate.mohp.gov.np/downloads/National%20Health%20Care%20Waste%20Standard%20Operating%20Procedure-2020.pdf

Ministry of Health Province Public Health Laboratory Koshi Province		Document Code: SOP-H-01
		Page No: Page 63 of 65
Title: Automated Haemotology Analyzer		Approved By: Director
Effective Date: 2081-04-01		
Prepared by: PPHL Koshi	Reviewed By:	

1. Introduction

Hematology analyzer is designed for simultaneous 18 parameter measurement. It provides quick counting and all operations are performed automatically. We only need to put the sampling nozzle into a sample container which contains whole blood and press the count switch. Result and date are displayed on a color LCD screen measured parameters are : WBC, RBC, HGB, HCT, MCV, MCH, MCHC, Platelets, Lymphocytes % , Mono %, Granulocytes %, Lymphocytes count, monocytes count, granulocytes count, RDW, PCT, RDW, PCT,MPV and PDW.

2. Principle

An electrolytic solution (Diluents) containing suspended blood cells is aspirated through the aperture. Two electrodes (on Internal & External) are located close to the aperture and constant current flows between them. When a blood cells passes through the aperture the resistance between the electrodes momentarily increases and a very small voltage change occurs corresponding to the resistance. The voltage signal in amplified and is send to the electronic circuit.

A threshold circuit eliminated signals caused by electrically noise dust debris and particles which are smaller or larger than blood cells.

To find the peak values, the signals are sent to A/D converter. The acquired data is stored in memory for each individual peak value. The data is corrected by the CPU and displayed on the screen

3. Instrument

Automated Hematology Analyzer (DXH 900 Beckman Coulter)

4. Procedure

1. Press the [Power] key on the front panel on.
2. Then press the power button of CPU Below
3. The power lamp light and the screen illuminates within 15-30 seconds. Cleaning the fluid path, printing and circuit self-check are automatically performed [when there is an error the “Red Light Illuminates in the Indicator above.
4. If Instrument powerups properly then do the daily checks by going to calendar menu in the task bar.
5. Once daily check has passed, the analyzer is ready for counting.
6. For single tube mode: To Aspirate the sample click the single tube presentation button (test tube sign) in the home screen then give sample Id and press OK then submit. Automatically the tube holder will protrude out.
7. Place the sample in the respective Sample holder, the instrument will automatically pull in the sample for analysis.
8. For auto loader mode: Place the samples with barcode in the sample rack and then keep the rack in sample buffer area.
9. The instrument will automatically take the rack inside for analysis.

10. All 18 parameter reports will be given within one minute and the report can be printed by giving the instrument print command manually. Transform the report in your Worksheet.
11. After the day's work is complete then
12. Turn off the power :
    - To turn the power off, press the power down key in the daily check menu. The analyzer automatically performs cleaning and the "after cleaning, the power will automatically shut off.
    - Then press the off button in the front panel of the instrument.

5. Calibration

Calibration is a procedure that confirms the accuracy of the analyzer and must confirm to guidelines established by the regulatory agencies. Calibration must be confirmed on a regular basis according to laboratory's standards and procedures.

Calibration in open mode (Venous blood mode) must be performed before pre dilution blood mode. If the calibrator is taken from a refrigerator wait at least 10 minutes for to reach room temperature.

1. On the MENU screen, press the QA key then press CBC Calibration button to display the CALIBRATION screen.
2. Put the XCAL sample vial in the rack the instrument will automatically do the CBC parameter Calibration.
3. For Differential Count Calibration LATRON calibrator solution must be assayed in similar way.
4. [After measurements the measured data and the "use this data for calibration?" message appears on the screen].
5. Check that there is no alarm or flag for the measure data and press the YES key to save the data. If there is an alarm, press the NO key and measure the calibrator again.
6. Press the OK key to display the CALIBRATION screen.
7. Perform the quality control on the QC screen with the hematology control and check the value with the control range.

6. Quality control

Quality control (QC) procedures are used to determine the accuracy and precision of the analyzer. These procedures allow the user to consistently, interpret laboratory data and ascertain acceptability of analysis results.

Quality control testing must be conducted according to established regulation and procedures in your particular state or country.

When a reagent lot number changed, change the internal components, new soft ware version installed it should be suggested the quality control be conducted.

The analyzer offers the following QC programs for monitoring and validating instrument performance

- X-R
- L and J [Levey and Jennings]
- XB
- XD-CV

These programs automatically calculate the plotting data from the sample data. The data can be displayed and printed as a table and graphs are plotted from the obtain data for each parameter for quality control.

There are three levels of hematology controls for each measured parameters - Low, Normal and High

Prior to running patient specimens run the three level control, the control data should be acceptable limits otherwise do not report the patient results

7. Maintenance

This analyzer has been designed to require minimal routine maintenance; the operator must routinely perform the scheduled maintenance procedures.

Maintenance Schedule:

Daily: Check reagent volume, printer paper reagent tube connection, data & time setting pump tube, touch screen, accuracy, measure hematology controls.

Every 200 counts: Do cleaning from Daily check page.

Periodically perform Shutdown procedure with cleaner

Monthly or Every 3000 counts: check sampling nozzles replace pump tubes.

To keep the analyzer in optimum condition, periodically check, clean and maintain it according to the above schedule.

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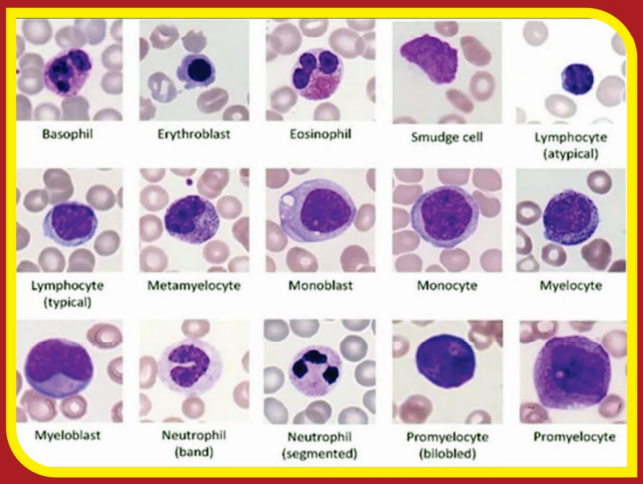
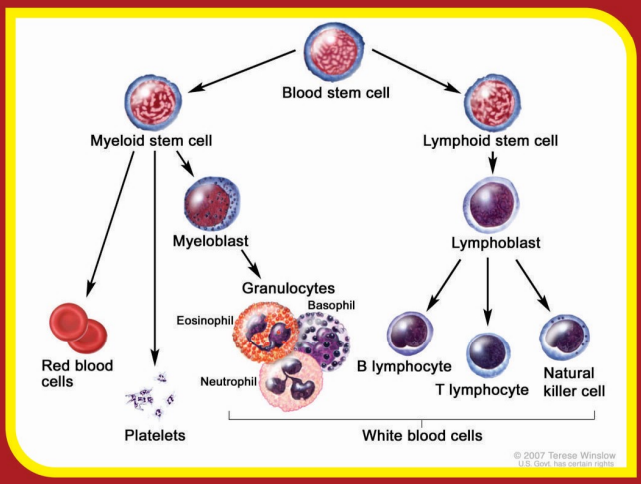
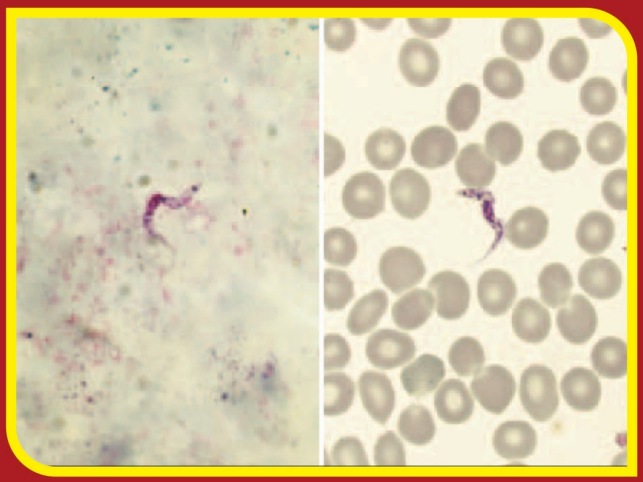
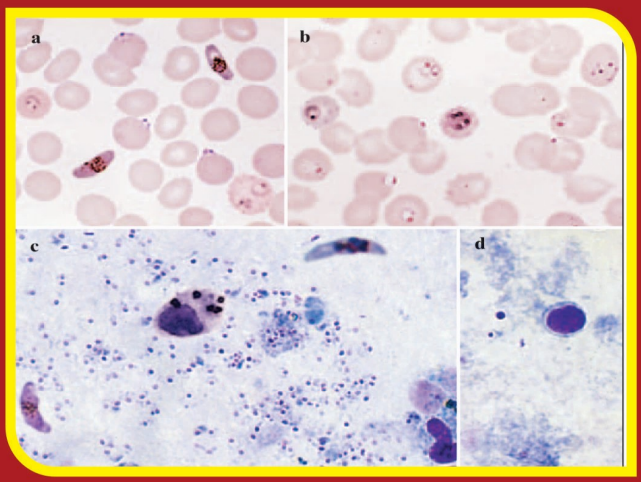








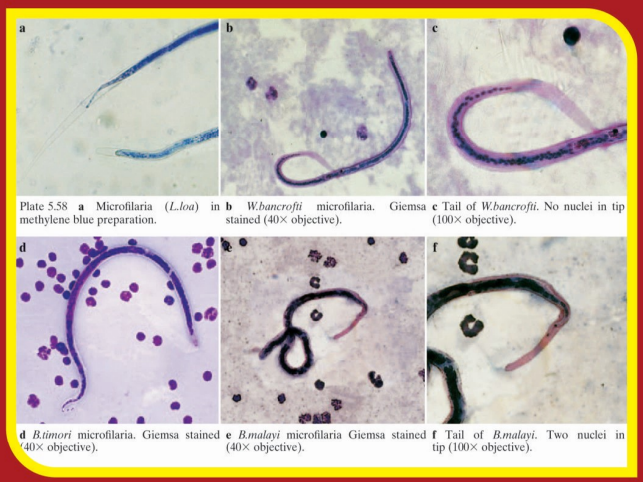
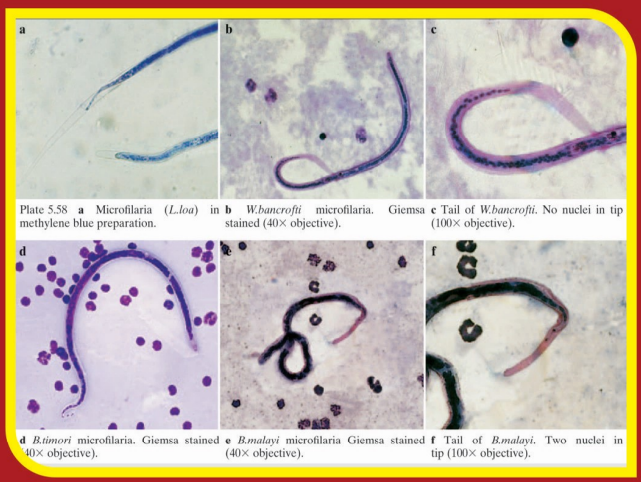


Plate 5.58 **a** Microfilaria (*L.loa*) in methylene blue preparation. **b** *W.bancrofti* microfilaria. Giemsa stained (40× objective). **c** Tail of *W.bancrofti*. No nuclei in tip (100× objective).

d *B.timori* microfilaria. Giemsa stained (40× objective). **e** *B.malayi* microfilaria Giemsa stained (40× objective). **f** Tail of *B.malayi*. Two nuclei in tip (100× objective).



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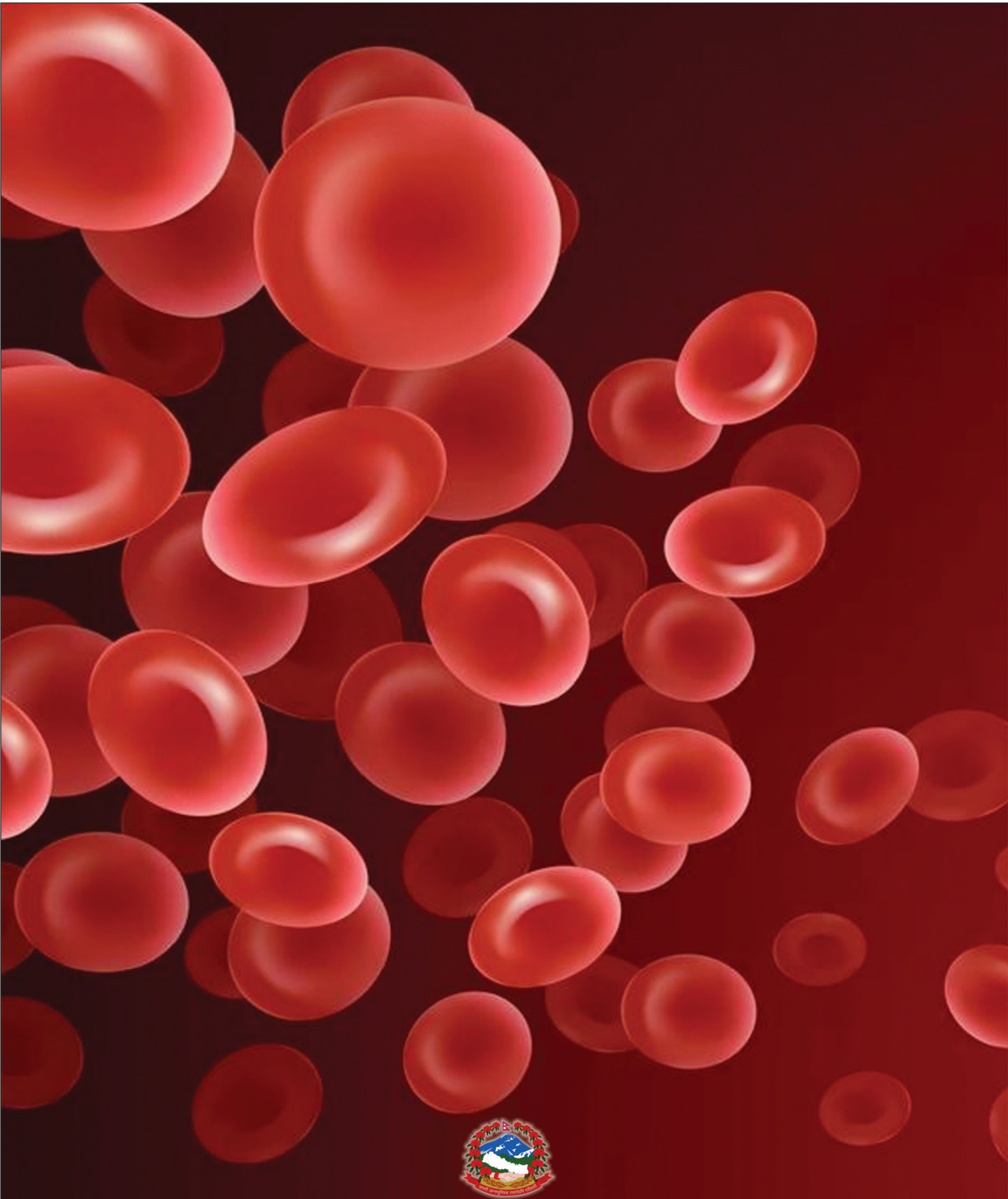

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