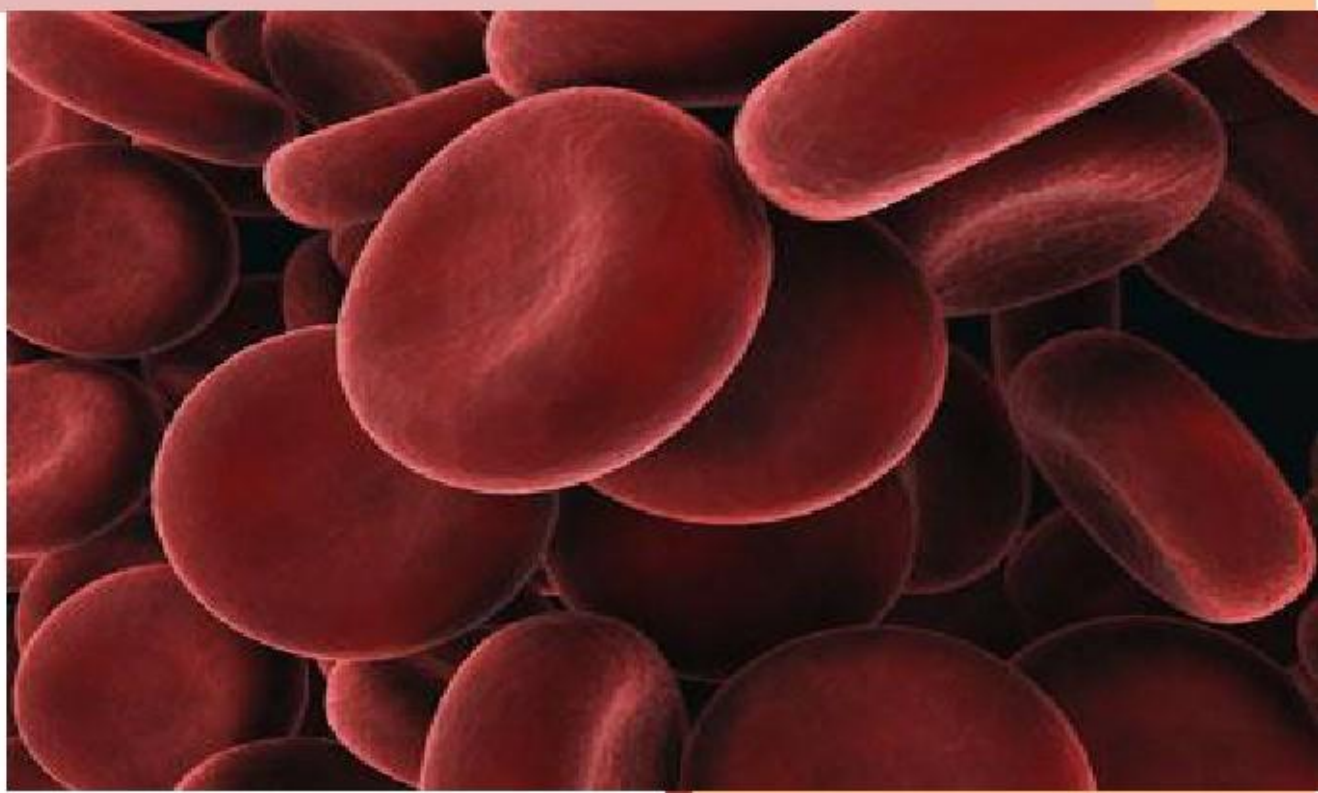
CLS 241

Practical Haematology



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Haematology Lab Manual

CLS 241

(Level Four)

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Contents

Blood Collection

There are two types of blood samples: venous blood and capillary blood.

Venous Blood

Veins are the most commonly used site for blood collection. The process of drawing blood from a vein is known as venipuncture. As a general rule, arm veins are the best source of blood. The preferred site is the antecubital fossa, which is where the arm bends at the elbow. The vein of choice is the median cubital vein. The cephalic and basilic can also be used. In unusual situations, it may be necessary to use hand or wrist veins when a suitable arm vein cannot be located (e.g. obese patients), or if the arms are bandaged or have been punctured repeatedly.

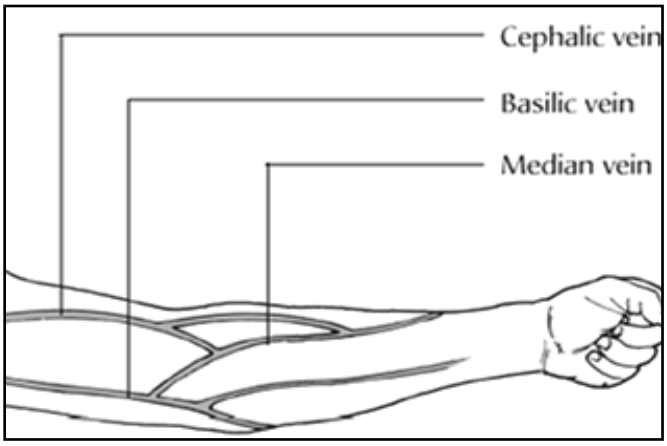

Figure 1: Common arm veins used for venipuncture.

Venipuncture Procedure

1. Ask the patient to make a loose fist and Select the appropriate vein for venipuncture.
2. Apply the tourniquet 3-4 inches above the collection site.
3. Clean the puncture site by making a smooth circular pass over the site with a 70% alcohol pad, moving in an outward spiral from the zone of penetration.
4. Uncap the needle with the bevel pointing upward.
5. Pull the skin tight with your thumb or index finger just below the puncture site.
6. Holding the needle in line with the vein, use a quick, small thrust to penetrate the skin and enter the vein in one smooth motion.
7. Holding the needle hub securely, insert the first vacutainer tube into the tube connector. Blood should flow into the vacuum tube.
8. After blood starts to flow, release the tourniquet and ask the patient to open his or her hand.
9. Once tube is full, use one hand to stabilize the needle in the vein and remove the tube with the other.
10. Insert other tubes in the correct order until all test samples are obtained.
11. Place a gauze pad over the puncture site and remove the needle.
    Immediately apply slight pressure, and then apply a fresh bandage over the penetrating site.

Needles Used for Venipuncture

- 19 or 21 gauge for adults.
- 23 gauge for children.

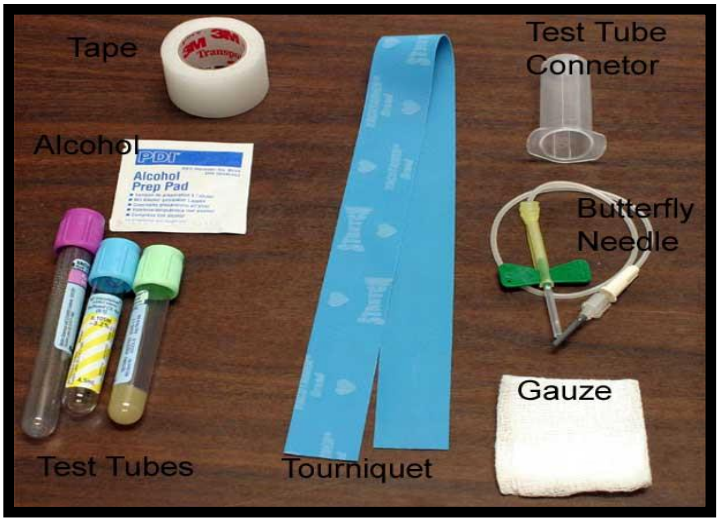

Figure 2: Common tools used for phlebotomy

Capillary Blood

Capillary blood is used for infants under 1 year of age and when it is not possible to obtain venous blood. Common sites for drawing capillary blood are the heel and the ball of the middle finger.

Skin puncture is carried out with a needle or lancet. In adults and older children blood can be obtained from a finger. The recommended site is the distal digit of the third or fourth finger on its palmar surface, about 3-5 mm lateral from the nail bed. Formerly, the earlobe was commonly used, but it is no longer recommended because reduced blood flow renders it unrepresentative of the circulating blood. In infants, satisfactory samples can be obtained by a deep puncture of the plantar surface of the heel in the area shown in figure 3. Because the heel should be very warm, it may be necessary to bathe it in hot water.

Capillary blood is free flowing, and thus it causes a greater risk of contamination and disease transmission. Venous blood is therefore more convenient for handling and it yields considerably more accurate results.

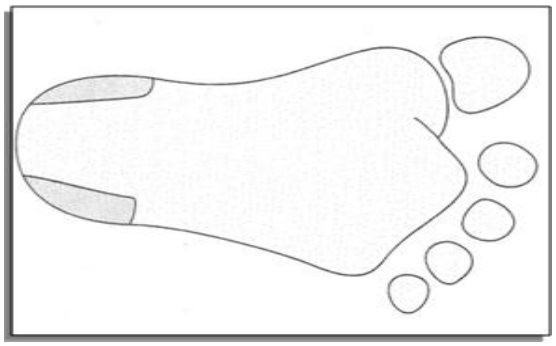

Figure 3: Skin puncture in infants. Puncture must be restricted to the parts of the heel indicated by the shaded area.

Collection of Capillary Blood

1. Clean the area with 70% alcohol and allow to dry.
2. Puncture the skin to a depth of 2-3 mm with a sterile lancet.
3. Wipe away the first drop of blood with dry sterile gauze.
4. Collect the second and following drops onto a reagent strip or by a 10 ml or 20 ml micropipette

Blood Parts

Blood is composed of two parts: a cellular part (blood cells) and a non cellular part called plasma.

Blood Cells

There are three types of blood cells: Red blood cells, white blood cells (leukocytes) and platelets.

Plasma

Plasma is the clear yellowish portion of blood. It contains fibrinogen, a coagulation factor that causes plasma to clot quickly. To obtain plasma, blood is collected into a tube with an anticoagulant.

Serum

Serum is the part of the blood that contains neither blood cells nor clotting factors. Serum is prepared by allowing the blood to naturally form a clot, and then using a centrifuge to remove the blood cells and the clot. The upper supernatant after centrifugation is the serum. To obtain serum blood should be collected into a sterile tube that contains no anticoagulants.

Figure4: Vacutainer tube for serum. It contains no anticoagulants

Anticoagulants

Whole blood is necessary for most haematological tests. Blood samples must be collected into tubes or bottles containing different anticoagulants to prevent coagulation (clotting). The anticoagulants commonly used are Ethylenediaminetetraacetic Acid (EDTA), Sodium Citrate and Heparin.

Ethylene-di-amine-tetra-acetic Acid (EDTA)

Ethylenediaminetetraacetic Acid is a chelating agent produced as a series of salts. The sodium and potassium salts of EDTA are powerful anticoagulants, and they are especially suitable for routine haematological work.

Chemical Action

EDTA acts by its chelating effect on the calcium molecules in the blood. It removes calcium ions, which are essential for coagulation.

Use

EDTA is the anticoagulant of choice for blood counts and blood films. It is also ideal for platelet counts as it prevents platelets from clumping. This anticoagulant, however, is not suitable for coagulation studies because it destroys clotting factors V and VIII.

Concentration

The recommended concentration of EDTA is 1.5 ± 0.25 mg/ml of blood. Excess of EDTA affects both red cells and leukocytes causing shrinkage and degenerative changes. A concentration exceeding 2 mg/ml of blood may result in a significant decrease in packed cell volume (PCV) and an increase in mean cell hemoglobin concentration (MCHC). The platelets are also affected; excess of EDTA causes them to swell and then disintegrate, leading to an artificially high platelet count. Therefore, it is important to ensure that the correct amount of blood is added and that the anticoagulant is thoroughly mixed in the blood added to it.

Figure 5: EDTA blood collection tube.

Trisodium Citrate

Chemical Action

Trisodium citrate removes free calcium ions by loosely binding to them forming a calcium citrate complex.

Use

This anticoagulant is used for coagulation studies and the estimation of the erythrocyte sedimentation rate (ESR).

Figure 6: Sodium citrate blood collection tube

Concentration

For coagulation tests, nine volumes of blood are added to one volume of the sodium citrate solution. For the ESR, four volumes of blood are added to one volume of the sodium citrate solution. Sodium citrate is available in two concentrations: 3.2% and concentrations: 3.2% and 3.8%.

Heparin

Heparin is a natural substance which is synthesized by the liver.

Chemical Action

Heparin neutralizes thrombin, an essential clotting factor, with the aid of a co-factor present in the albumin fraction of plasma.

Use

Heparin is an effective anticoagulant and it does not alter the size of red cells. It serves as a good dry anticoagulant when it's important to minimize the chance of lysis after blood has been withdrawn. Heparin is the best anticoagulant for osmotic fragility and is suitable for immunophenotyping.

Heparinized blood is not recommended for cell counting because of its clumping effect on platelets and leukocytes. It also should not be used for making blood films because it gives a faint blue coloration to the background when Romanowsky dyes are used. It inhibits enzyme activity, and it is not suitable for use in the study of polymerase chain reaction (PCR) with restriction enzymes.

Concentration

The heparin concentration used is 10-20 international units (IU) per Ml of blood.

Figure7: Heparin Blood Collection

Haemoglobinometry

Hemoglobin is an iron containing protein molecule found in red blood cells which transports oxygen from the lungs to the body's tissues. Four polypeptide chains (globins), each wrapped in a specific way around its own heme group, make up the hemoglobin molecule. A heme group consists of an iron atom in the ferrous state (Fe^{2+}) and a porphyrin ring. There are three kinds of normal hemoglobin molecules: hemoglobin A ($\alpha_2\ \beta_2$), hemoglobin A_2 ($\alpha_2\ \delta_2$) and hemoglobin F ($\alpha 2\ \gamma 2$). The hemoglobins present in healthy adults are: Hb A (96-98 %), Hb A_2 (1.5- 3.2%) and Hb F (0.5 -0.8%). In fetuses, the major Hb is F.

It is possible to use manual, semiautomated, or automated techniques to determine hemoglobin concentration and other blood components. Manual techniques are generally low cost with regard to equipment and reagents but are labor intensive. Automated techniques entail high capital costs but permit rapid performance of a large number of tests by a smaller number of laboratory workers. Automated techniques are more precise, but their accuracy depends on correct calibration and the use of reagents that are usually specific for the particular analyzer. Many laboratories now use automated techniques, certain manual techniques are necessary as reference for standardization of the methods.

Measurement of Hemoglobin (Hb) concentration in a whole blood sample is a basic screening test for anemia and polycythemia. The hemoglobin concentration of the solution may be estimated by measurement of it color, by its power of combining with oxygen or carbon monoxide, or by its iron content. The methods mostly used are color or light-intensity matching techniques. Ideally, for assessing clinical anemia, a functional estimation of hemoglobin should be carried out by measurement of oxygen capacity, but this is hardly practical in the routine hematology laboratory. It gives results that are at least 2% lower than those given by the other methods. The iron content of hemoglobin can be estimated accurately, but again the method is impractical for routine use. Estimations based on iron content are generally taken as authentic, iron bound to inactive pigment is included.

Haemiglobincyanide (Cyanmethaemoglobin) Method

The haemiglobincyanide (Cyanmethaemoglobin) method is the internationally recommended method for determining the hemoglobin concentration of blood.

Principle

Blood is diluted in Drabkin's solution, a solution containing potassium cyanide and potassium ferricyanide. Potassium ferricyanide oxidizes hemoglobin to methaemoglobin. Methaemoglobin then combines with potassium cyanide to form Cyanmethaemoglobin (HiCN). The absorbance of the solution is measured in a spectrophotometer at a wave length of 540 nm against Drabkin's solution as a blank. The result is calculated using the formula provided below and it is expressed in gm/dl.

Test Sample

Venous or capillary blood collected into an EDTA tube. Alternatively, free flowing capillary blood may be added directly to the diluting fluid and measured.

Procedure

1. Pipette 4ml of Drabkin's solution into a test tube.
2. Pipette 20 µl (0.02 ml) of properly mixed blood.
3. Clean outside of pipette and wash out the blood in the tube containing the diluent.
4. Mix and leave for 5-10 minutes for the reaction to complete.
5. Using drabkin's solution as a blank, read the absorbance in the spectrophotometer at wavelength 540 nm.

Calculation

Use the following formula:

$$\text{Hb in g/dl} = \frac{A \times 64500 \times DIL}{44 \times D \times 1000 \times 10} = A \times 29.3$$

Where: A = Reading of absorbance of Hb solution
64500 = Molecular wt. of Hb
44 = Millimolar extinction coefficient
D = Thickness of cuvette
1000 = Conversion factor of mg to gm
DIL = Dilution Factor = 200

Notes on Technique:

- The blood sample must be properly mixed before sampling and allowed to warm
- Care should be taken when handling potassium cyanide.
- Use clean tubes and pipettes.

Comments

The cyanmethaemoglobin method is the reference method for Hb estimation because: **a)** all Hb forms except sulphaemoglobin are estimated, **b)** highly reliable and stable reagents are available and **c)** the method can be easily standardized.

Normal Ranges

Adult males: 13 – 17 g/dl
Adult females: 11.5 – 16.5 g/dl
Newborns and infants: 14 – 22 g/dl

Oxyhaemoglobin Method

The HbO2 method is the simplest and quickest method for general use with a photometer. Its disadvantage is that it is not possible to prepare a stable HbO_2 standard, so the calibration of these instruments should be checked regularly using HiCN reference.

Direct Reading Portable Haemoglobinometers

Color Comparators

These are simple clinical devices that compare the color of blood against a range of colors representing hemoglobin concentrations. They are intended for anemia screening in the absence of laboratory facilities.

Portable Hemoglobinometers

Portable hemoglobinometers have a built-in filter and a scale calibrated for direct reading of hemoglobin in g/dl or g/l. They are generally based on the HbO_2 method. Some hemoglobinometers require dilutions of blood and others do not require dilutions because blood is drawn into cuvettes containing certain chemicals.

Noninvasive Screening Tests

Methods are being developed for using infrared spectroscopy at body sites, mainly a finger, to identify the spectral pattern of haemoglobin in an underlying blood vessel and derive a measurement of haemoglobin concentration.

Automated Haemoglobin measurement

Most automated counters measure haemoglobin by a modification of the manual HiCN method with cyanide reagent or with a nonhazardous chemical such as sodium lauryl sulphate, which avoids possible environmental hazards from disposal of large volumes of cyanide containing waste. Modifications include alterations in the concentration of reagents and in the temperature and pH of the reaction. A nonionic detergent is included to ensure rapid cell lysis and to reduce turbidity caused by cell membranes and plasma lipids. Measurements of absorbance are made at a set time interval after mixing of blood and the active reagents, but before the reaction are complete.

Haematocrit Determination (Packed Cell Volume-PCV)

The heamatocrit (PCV) is the percentage of the volume of blood occupied by red cells. It is a screening test for anemia or polycythemia. When accurate measurements of hemoglobin and rell cell counts are available, the absolute values for red cells can be calculated.

Manual Haematocrit Determination

A volume of anticoagulated or capillary blood is placed in a glass tube. The glass is then centrifuged so that the blood is separated into its main components: red cells, white cells, platelets and plasma. Ideally there should be complete separation of cells and plasma. Haematocrit is the ratio of the height of the red cell column to that of the whole blood sample in the tube.

The two methods currently used for direct measurement of PCV are:

1. Macro- method using Wintrobe tubes
2. Micro- method using capillary tubes

The micro-method is more popular because: a) centrifugation lasts for a shorter time with this method and b) it results in better packing of the red cells.

Micro-hematocrit Method

Test Sample

Anticoagulated venous blood or capillary blood

Equipment

1. Micro haematocrit centrifuge
2. 75 mm long capillary tubes with an internal diameter of 1 mm.
3. Plastic sealer or Bunsen burner

Procedure

Blood samples should be as fresh as possible and well mixed.

1. Using a capillary tube, allow blood to enter the tube by capillary action stopping at 10-15 mm from one end. Wipe the outside of the tube.
2. Seal the dry end by pushing into plasticine two or three times. If heat sealing is used, rotate the dry end of the tube over a fine Bunsen burner flame.
3. Place the tube into one of the centrifuge plate slots, with the sealed end against the rubber gasket of the centrifuge plate. Keep a record of the patient number against the centrifuge plate number.
4. Centrifuge for 5 minutes.
5. Read the PCV in the micro haematocrit reader. The haematocrit result is expressed in either a percentage or liter per liter (l/l).

Note:
It is preferable to the test in duplicate.

Normal Ranges
Adult males = 0.40 – 0.52 (40% - 52%)
Adult females = 0.37 – 0.47 (37% - 47%)

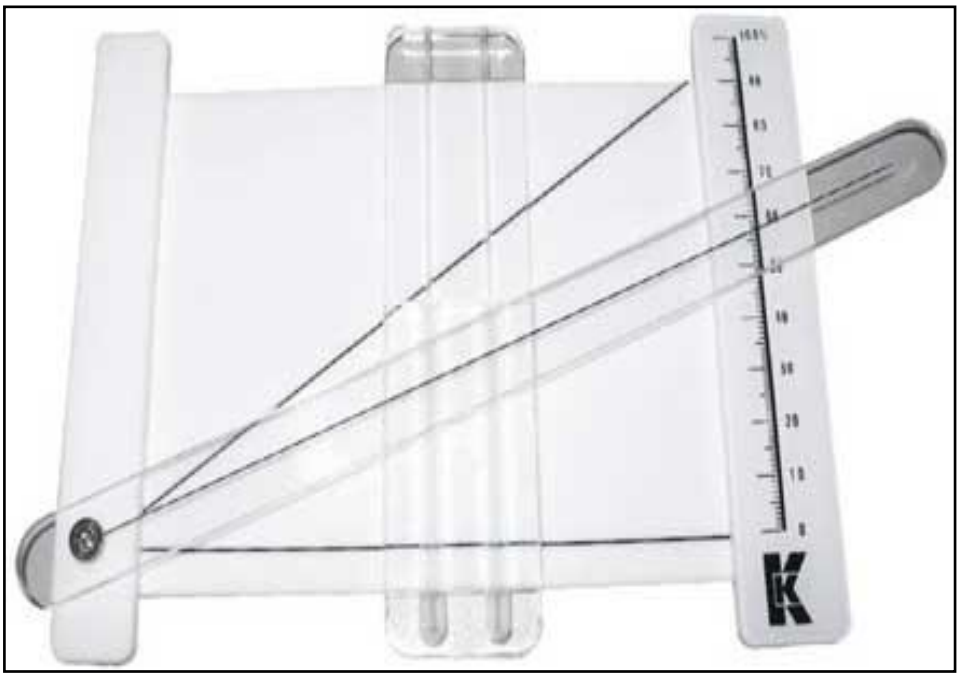

Figure 8: Micro-haematocrit Reader.

Automated PCV Measurement

Modern automated blood cell counters estimate PCV by technology that doesn't involve packing red cells by centrifugation. For this reason, the International Council for Standardization in Haematology has suggested that the term haematocrit rather than PCV should be used for automated measurement. With automated instruments, the derivation of the RBC, PCV and MCV are closely interrelated. The passage of a cell through the aperture of an impedance counter or through the beam of light of a light- scattering instrument leads to the generation of an electrical pulse. The number of pulses generated allows the RBC to be determined. Pulse high analysis allows either the MCV or the PCV to be determined. If the average pulse height is computed, this is indicative of the MCV. The PCV can be derived from the MCV and RBC. Similarly, if the pulse heights are summated, it is indicative of the PCV. The MCV can, in turn be calculated.

Red Blood Cell Count

A red blood cell count is typically ordered as part of a complete blood count (CBC). It is used as a screening test for anemia and polycythemia.

Manual Red Blood Cell Count (Using Formal Citrate as a Diluent)

Principle

A suitable dilution of blood (1/200) is made in formal citrate solution. This diluent lyses the white cells leaving the red cells intact. The number of RBCs in $1/5^{th}$ of the RBC square is counted using an improved Neubauer counting chamber called a hematocytometer. The hematocytometer consists of 9 counting squares with each square having an area of $1mm^2$. Every square is filled with 0.1 ml of diluted blood. The result in expressed as RBCs/ lit of blood.

Procedure

1. Pipette 4 ml of RBC diluents in a tube.
2. Pipette 20 µl of well mixed blood into the tube containing the diluents.
3. Clean outside of pipette and wash out the blood in the tube containing the diluent (dil 1/200).
4. Mix the contents of the tube and fill the counting chamber with the diluted blood (0.1 ml of diluted blood in each square).
5. Leave on bench for 2-5 minutes for the cells to settle.
6. Count the RBCs under the microscope using the (x40) objective lens and lowering the condenser. The RBCs are counted in $1/5^{th}$ of the RBC square i.e. 0.2 mm^2.

Calculations

N = the number of cells in 0.02 ml of diluted blood (the number of cells in five squares)

Number of cells in 1 ml of diluted blood = $\frac{N}{0.02\ \mu l} = \frac{N \times 100}{2} = N \times 50$

Number of cells in 1 ml of whole blood = N x 50 x 200 = N x 10000

Normal Ranges

- Men: $5 \pm 0.5 \text{ X } 10^{12}/\text{l}$
- Women: $4.3 \pm 0.5 \text{ X } 10^{12}/\text{l}$

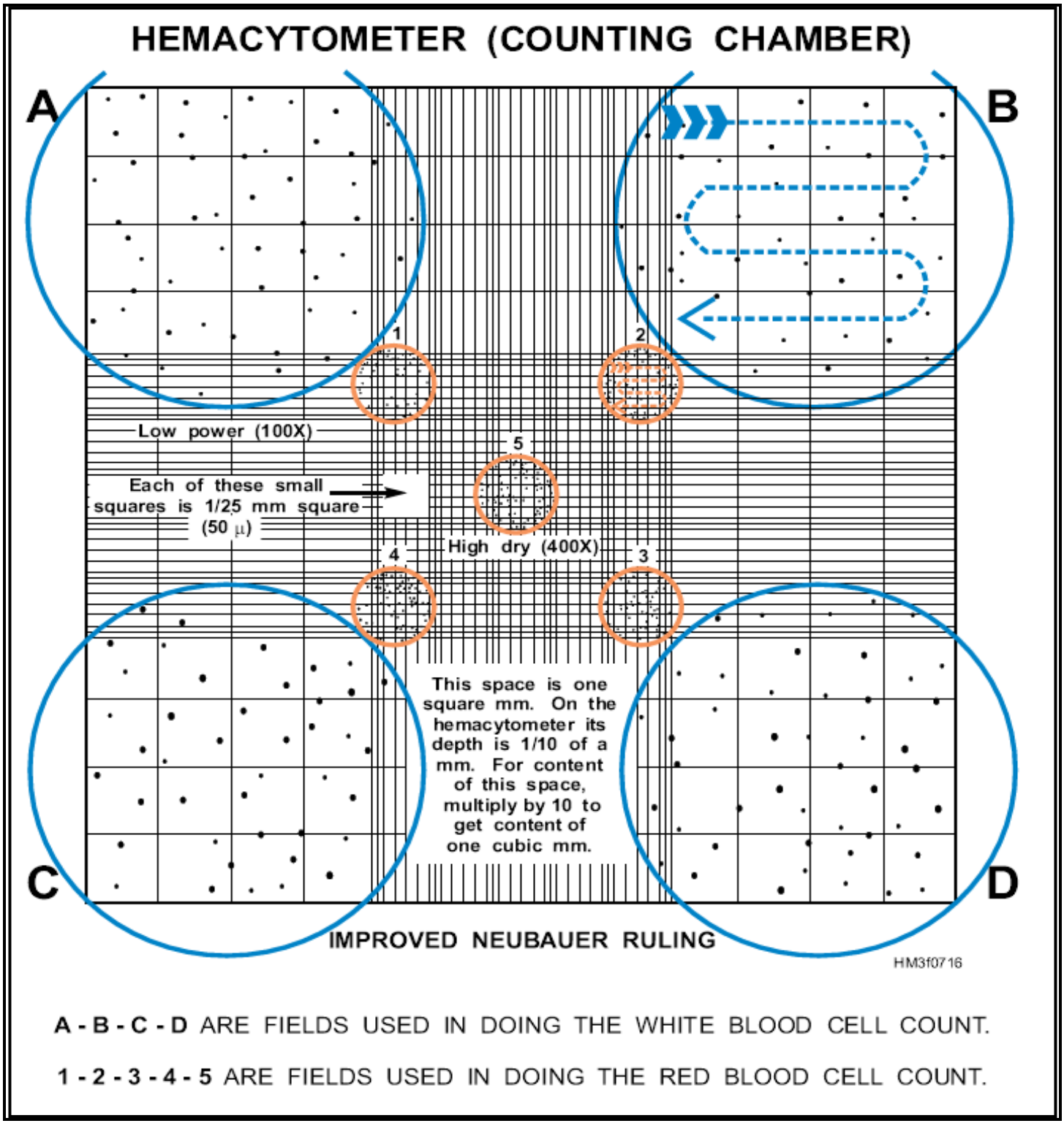

Figure 9: Illustration showing different parts of the counting chamber.

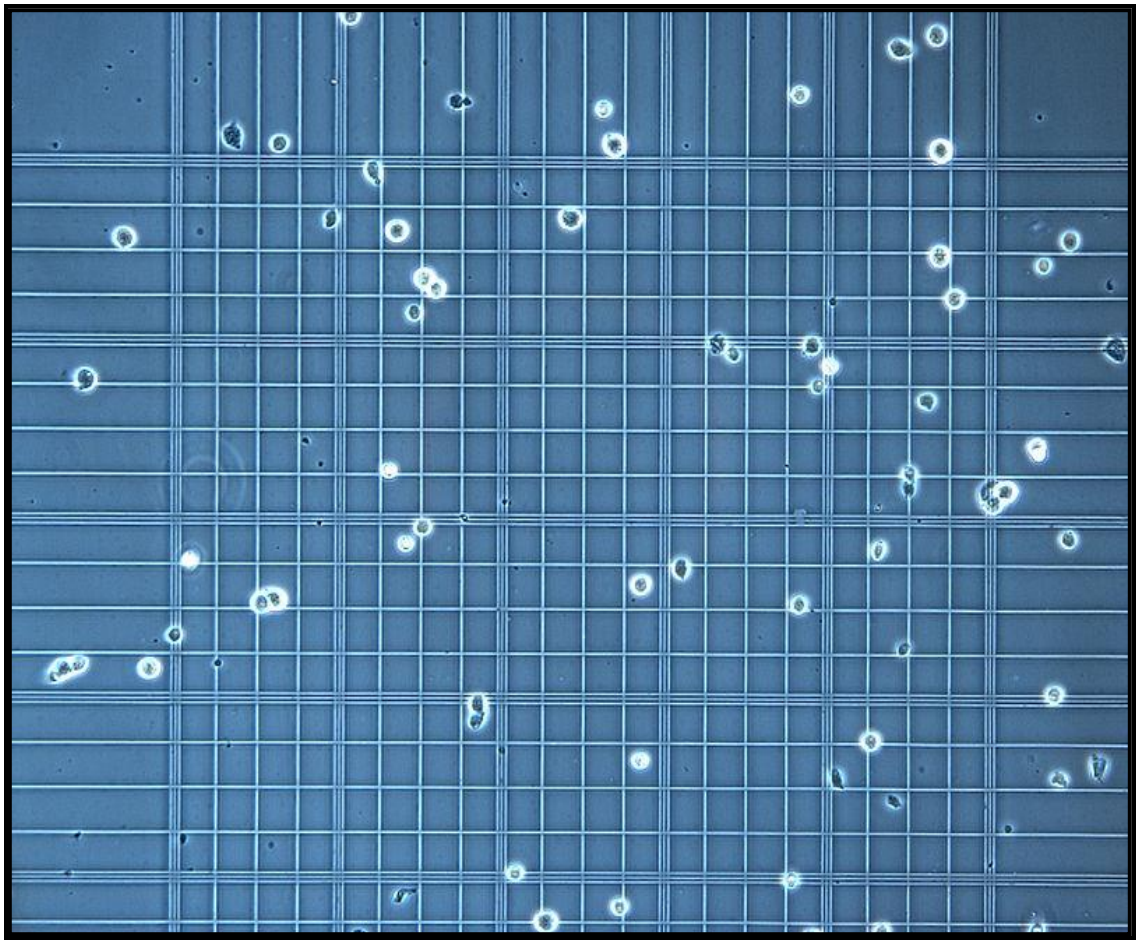

Figure 10: Appearance of red blood cells on the counting chamber under the microscope.

Automated Red Cell Count

Red cells and other blood cells can be counted in systems based on either aperture impedance or light-scattering technology. Because large numbers of cells can be counted rapidly, there is a high level of precision. Consequently, electronic counts have rendered the RBC of much greater clinical relevance than was possible when only slow and imprecise manual RBC count was available.

Red Cell Indices

The calculation of the size and haemoglobin content of the red cells from the Hb, PCV and red cell count, have been widely used in the classification of anemia. The three most common indices are the MCV, the MHC and the MCHC.

Mean Corpuscular Volume (MCV)

The MCV is the average volume of a single red cell expressed in femtoliters (fl) or 10^{-15} L. It helps in determining the size of the RBC. The PCV and red cell volume are used in its calculation.

Calculation Formula

$$MCV = \frac{PCV\ \% \times 10}{\text{RBC count (the number of millions not the actual count)}}$$

Example:
PCV = 0.45 L/L (45%), **RBC** = 5 x 10^{12}/L

$$MCV = \frac{PCV \times 10}{RBC} = \frac{45(\%) \times 10}{5} = 90\ fl$$

Reference Range

The MCV reference range is 83-101 fl. The MCV is increased in macrocytic anemias (e.g. megaloblastic anemia) and decreased in microcytic anemias (e.g. iron deficiency, thalassemia)

MCV value is within the normal range → Normocytic.
MCV value is above the normal range → Macrocytic.
MCV value is below the normal range → Microcytic.

Mean Cell Hemoglobin (MCH)

MCH is the average weight in picograns of Hb in one red cell. The Hb level and the RBC count are used for calculation.

Calculation Formula

$$MCH = \frac{Hb\ (g/dl) \times 10}{\text{RBC (use the number of millions rather than the actual count)}}$$

Example:
Hb = 150g/l (15 g/dl), **RBC** = 5 x 10^{12} /L

$$MCH = \frac{15(g/dl) \times 10}{5} = 30\ pg$$

Reference Range

The MHC reference range is 27-32 pg. MHC is increases in macrocytic anemia and decreased in microcytic anemias.

MCH value is within the normal range → Normochromic
MCH value is above the normal range → Hyperchromic
MCH value is below the normal range → Hypochromic

Mean Cell Hemoglobin Concentration (MCHC)

The MCHC is the concentration of haemoglobin per unit volume of red blood cells expressed as a percentage, g/dl or g/l. Hemoglobin and PCV are required to calculate MCHC.

Calculation Formula

$$MCHC = \frac{Hb(g/dl)}{PCV\%} \times 100$$

Example:
If one liter of blood contains 0.45 liters of packed cells and 150g of Hb (150g Hb are contained in 0.45 liters of RBCs). What is the Hb concentration?

Hb = 15 g/dl (150 g/l), **PCV** = 45% (0.45 l/l)

$$MCHC = \frac{Hb\ (g/dl)}{PCV\%} \times 100 = \frac{15}{45} \times 100 = 33.3\% \text{ or } 33.3\ g/dl = 333.3\ g/l$$

Reference Range

The reference range for MCHC is 32-36 g/dl or (320-360 g/l)**.**

A fully saturated red cell has a hemoglobin concentration of 36 g/dl. MCHC is a useful guide to the degree of hypochromia present in iron deficiency anemia. The Hb and PCV can be estimated reasonably accurately and the derived MCHC is therefore a reliable parameter.

Automated Estimation of Red Cell Indices

In automated counters, MCV is measured directly, but in semiautomated counters MCV is calculated by dividing the PCV by RBC. MCH is derived from the Hb divided by RBC. The MCHC is derived from the Hb and the PCV with instruments that measure the PCV and calculate the MCV, whereas when the MCV is measured directly and the PCV is calculated, the MCHC is derived from the Hb, PCV and RBC.

Blood Film Preparation and Staining

Blood and bone marrow films that are well prepared and properly stained have a great value in haematology. They are used in: a) viewing cell morphology for diagnosing anemias and leukemias, b) WBC differential counts and c) estimating the number of platelets.

Technique of Spreading

Spreading is done on dust free glass microscope slides using a spreader, which is a microscope slide having one corner removed at each end.

Types of Blood Films

(1) Thick blood film which is used for parasitology e.g. malaria. It is prepared by handling the spreader by the edge, using the corner to spread the blood in a circular form with 3-6 movements.
(2) Thin blood film which is used in hematology and prepared as described below.

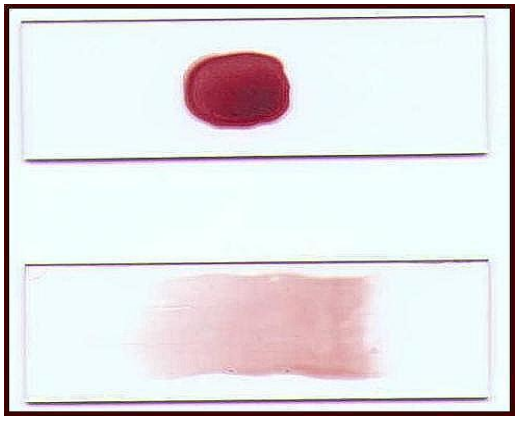

Figure 11: Picture showing thick (top) and thin (bottom) blood films.

Blood Smear Preparation Method

1. EDTA anticoagulated blood is used. Blood is taken using capillary tubes.
2. Place a drop of blood near the end of the slide, about 1-2 cm from one end.
3. Place the spreading slide at an angle of 45◦ about 1 cm in front of the drop of blood.
4. Back the spreader into the drop of blood. The spreader catches the blood and it spreads by capillary action along its edge.
5. Maintaining the 45◦ angle, push the spreader smoothly across the slide. This pulls the blood across to makethe smear. It should take about one second to make the smear.
6. Allow the film to air dry.

Notes on method:

- The edge of the spreader must be very smooth, and narrower than that of the slide.
- The spreader must be cleaned and dried if it had been used for spreading more than five films.
- A smooth action is required, with the edge of the spreader held against the slide.
- Films may be prepared manually or automated slide spreaders.
- The blood film length shouldn't be too long nor should it be too short.
- All slides should be labeled for identification.

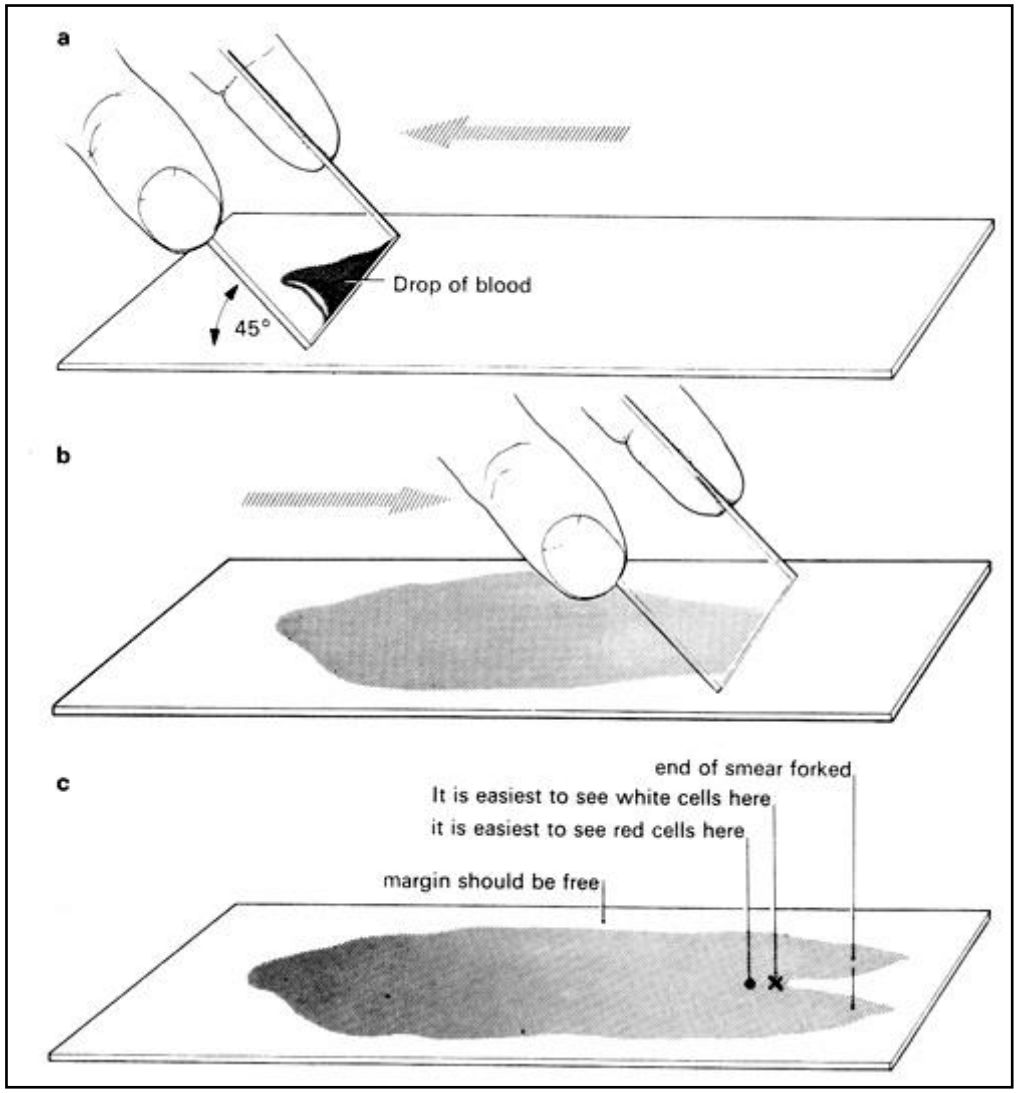

Figure 12: Illustration showing the steps of making a blood smear

Types of Stains

Romanovwsky dyes are universally employed for routine staining of blood films. Romanowsky stains include leishman's stain, write stain, may grunwald stain, jenner's stain and giemsa stain. Romanowsky dyes consist of two components:

(1) **Basic Part:** azure blue or methylene blue which bind to the acidic part of the cell (nucleus) and stain it blue.
(2) **Acidic Part:** eosin Y which binds to the basic parts of the cell (proteins, cytoplasm and Hb) giving them a red color.

Azure B(basic) → nuclei (containing nucleic acid) → **Blue**

Azure B(basic) → Basophilic granules (containing heparin which is acidic) →**Violet**

Eosin Y(acidic) → Cytoplasm and Hb (basic) → **Red**

Eosin Y(acidic) → Eosinophilic granules (alkaline) → **Red orange granules**

Staining Steps

1. Fixation of blood cells to protect them from hemolysis due to washing. Well-fixed cells resist the action of water. Fixation is done by:
   - Methanol (1.5 min)
   - B-Undiluted stain (neat stain) as in leishman's & wright stain
2. Staining
3. Washing

Manual Staining Method

Leishman's and Write Method:

1. Place dried film on the staining rack with film facing upwards.
2. Flood slide with neat stain (1 volume of Pasteur pipette)**.**
3. Allow to stain for 5 min.
4. Add double volume (2 volumes of Pasteur pipette) of the buffer pH 6.8 on the stain. Do not wash.
5. Allow to stain for 10-15 min.
6. Wash gently with distilled water.
7. Clean underneath the slide and leave to dry.

Automated Blood Film Makers

In most haematology laboratories today, automated blood film makers and strainers are used. Studies are still being conducted to compare the slides made by automated slide makers to those that are made manually. The manufacturer's instructions should be followed unless local experience has demonstrated that variation of the recommended technique achieves better results.

Red Blood Cell Morphology in Health and Disease

Normal RBCs are minute biconcave discs. However, on well spread and well stained films, they appear as reddish brown round smooth discs with a central pallor. They have a diameter of 6-8.5 micrometers.

Normal and diseased red cells are subject to considerable distortion during spreading of films. It is therefore very important to scan films carefully and select an area of minimal distortion for examination. Red blood cells should be examined in an area with little or no rouleaux formation. Thus, technologists always avoid thick areas where rouleaux have formed, and very thin areas where RBCs are maximally distorted.

Notes:

- A normal RBC has a normal size (normocytic) and a normal Hb content i.e. normal color (normochromic).
- Red cells are stained by the eosin component of romanovwsky dyes

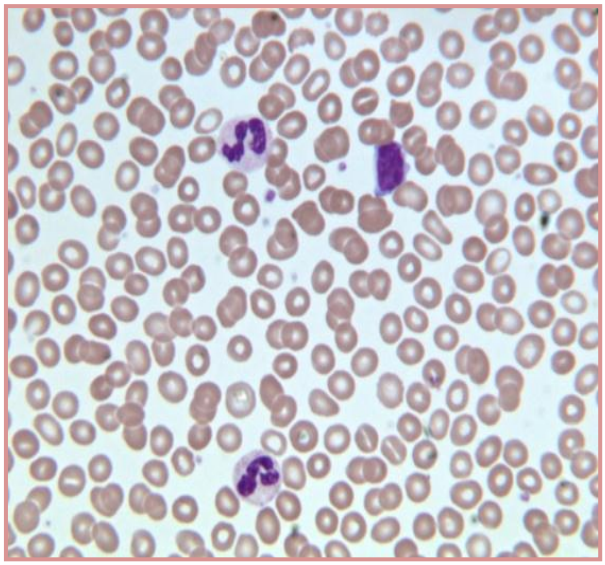

Figure 13: Normal peripheral blood film**.** Red cells are round, normocytic and normochromic.

Causes of Variation in RBC Morphology

Variation in RBC morphology stems from four main reasons:

(a) Abnormal erythropoisis
(b) Inadequate Hb synthesis
(c) Damage to, or changes affecting RBCs after leaving the bone marrow
(d) Attempts of the bone marrow to compensate for anemia

Types of Variation in RBC Morphology

The four causes mentioned above give rise to the following abnormalities of the red cells:

Variation in Size (anisocytosis): → **Macrocytosis** :RBC larger than normal
→ **Microcytosis** : RBC smaller than normal

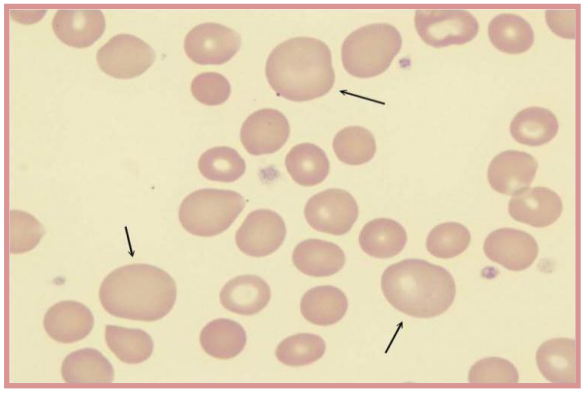

Figure 14: Blood film showing Macrocytes

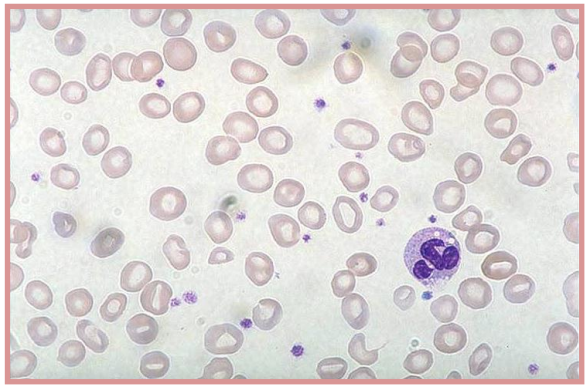

Figure 15: blood film showing microcytic-hypochromic cells

Variation in Shape (poikilocytosis)

- **Tear-drop cells:** cells shaped like tear drops or pears.
- **Elliptocytes**: they can vary in appearance from oval-shaped (Ovalocytes) to thin pencil-shaped forms (Pencil cells).
- **Keratocytes:** also described as **helmet cells** or **bite cells.** These cells are fragmented red blood cells that have been "scooped out" so they resemble helmets or appear as if they have been bitten. These "bites" result from either the removal of a heinz body by the phagocytic function of the spleen, or from mechanical damage.
- **Schistocytes**: RBC fragments.
- **Echinocytes**: also known as "crenated cells" and "burr cells". These cells have many tiny spicules (10-30) evenly distributed over the cell membrane. Although often confused with acanthocytes, the projections on echinocytes are much more uniform in shape and distribution in.
- **Acanthocytes:** also referred to as "spur cells". Spheroid RBCs with few large spiny projections. 5-10 spicules, irregular spacing and thickness (must be differentiated from echinocytes).
- **Sickled cells:** banana-shaped or crescent-shaped cells
- **Blister cells:** RBCs with vacuoles or markedly thin areas at periphery of membrane**.**
- **Pyknocytes:** irregularity contracted cells.

Variation in Hemoglobin Content

- **Hypochromasia**: color is paler than normal.
- **Anisochromasia** : cells having a dimorphic picture.
- **Target cells:** Thin, hyopochromatic cell. Round area of central pigmentation**.** also called codocytes.
- **Leptocytes** : very thin cells with a colorless central part.
- **Spherocytes:** sphere-shaped cells with deep coloration and no central pallor.
- **Stomatocyte:** also called fish mouth cell. Uniconcave RBC, slitlike area of central pallor.

Other Abnormalities

- **Late normoblast**: an immature nucleated RBC with a basophilic nucleus.
- **Polychromasia**: cells with pale green or blue coloration, which is a sign of immaturity.
- **Howel jolly bodies:** nuclear remnants that appear as Small (1 mm), round, dense, purple bodies in RBCs.
- **Basophilic stippling:** Fine, medium, or coarse blue granules uniformly distributed throughout RBC.
- **Pappenheimer bodies:** small peripherally sited basophilic inclusions. They are composed of hemosiderin. They appear purple in color with conventional staining. Using perl's stain gives them a blue color.
- **Malarial parasite:** stages of different malarial species.
- **Heinz bodies:** oxidized and denatured Hb. Stained by supravital stains (new methylene blue, brilliant cresyl blue).

Red Cell Abnormality	Associated Conditions
Macrocyte	Megaloblastic anemia, liver disease, alcoholism
Microcyte	Hemoglobinopathies, iron deficiency anemia
Tear drop cells	Extra medullary hemopoisis
Elliptocytes	Hereditary Elliptocytosis
Ovalocytes	Megaloblastic anemia
Schistocytes	Disseminated intravascular coagulation(DIC), burns, heart valve diseases
Sickled cells	Sickle cell anemia
Echinocytes	Sever liver disease
Acanthocytes	Liver disease, renal disease, post-splenectomy, alcoholic cirrhosis, hemolytic anemia, thalassemia
Pencil cells	Iron deficiency anemia
Target cells	Chronic liver disease, iron deficiency anemia, thalassemia, post splenectomy
Blister cells	G6PD deficiency. Other oxidant stress.
Helmet cells, Bite cells	G6PD deficiency, DIC, heart valve disease
Spherocyte	Hereditary spherocytosis
Stomatocyte	Liver disease, alcoholism
Leptocyte	Sever iron deficiency, thalassemia
Nucleated RBCs	Thalassemia, acute bleading
Basophilic stippling	Megaloblastic anemia
Howell-jolly bodies	Postsplenectomy
Pappenheimer bodies	Iron overload and hyposplenism
Heinz bodies	G6PD deficiency,

Table 1: showing some common RBC abnormalities and their associated conditions.

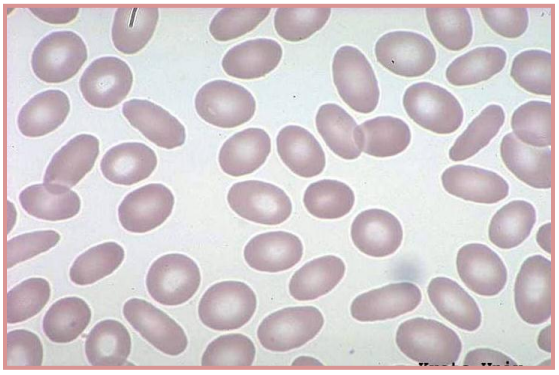

Figure 16: Elliptocytes. blood film showing elliptocytes.

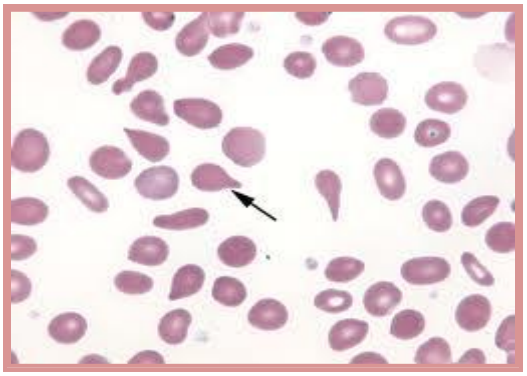

Figure 17: blood film showing tear-drop cells

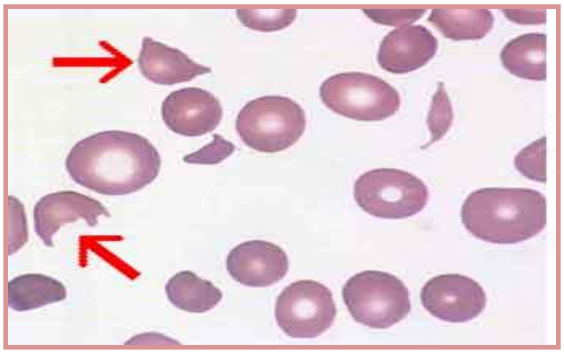

Figure 18: blood film showing some helmet cells.

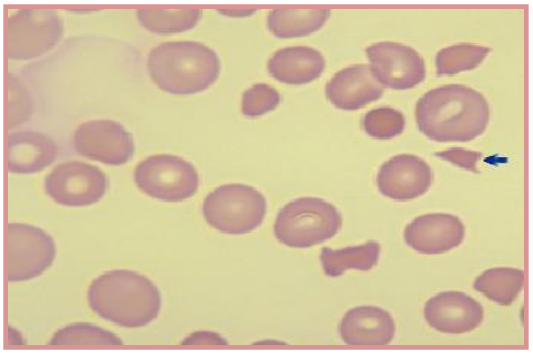

Figure 19: blood film showing schistocytes (fragmented RBCs)

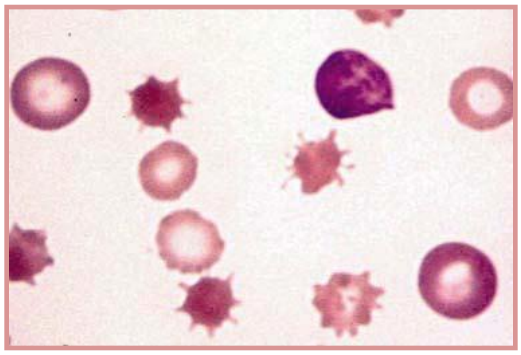

Figure 20: Blood film showing some acanthocytes.

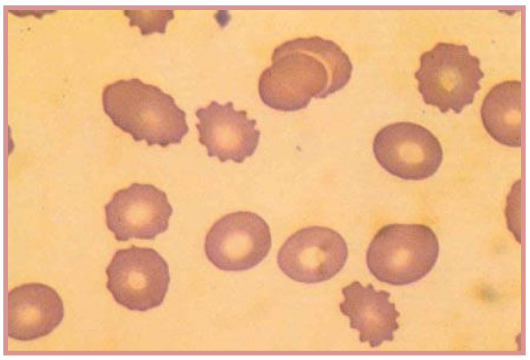

Figure 21: Blood film showing some echinocytes.

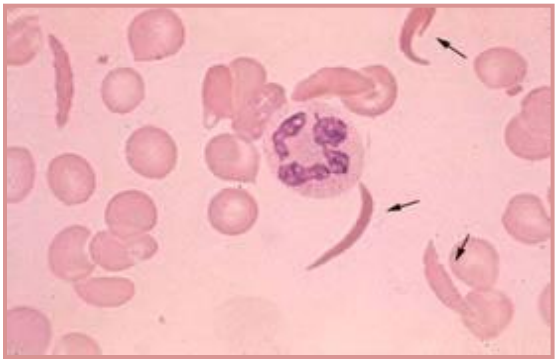

Figure 22: Blood film showing some sickled cells.

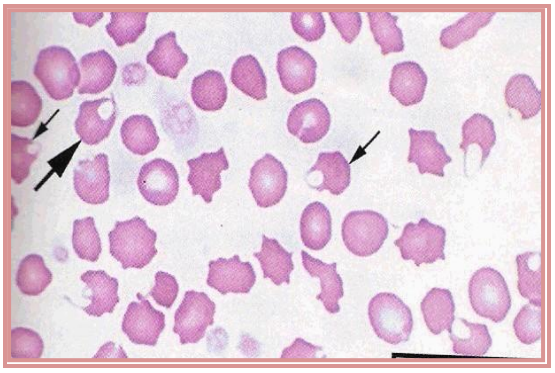

Figure 23: Blood film showing some blister cells

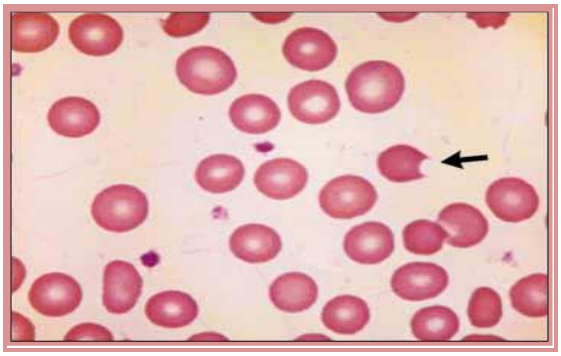

Figure 24: Blood film showing a bite cell.

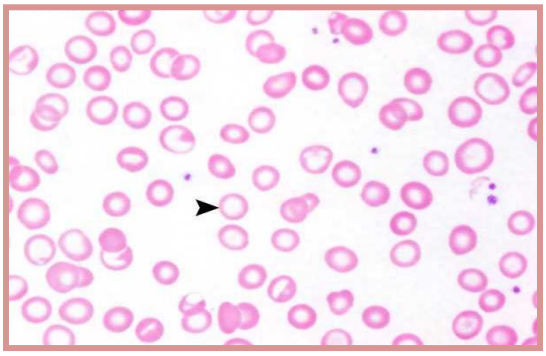

Figure 25: Blood film demonstrating hypochromasia

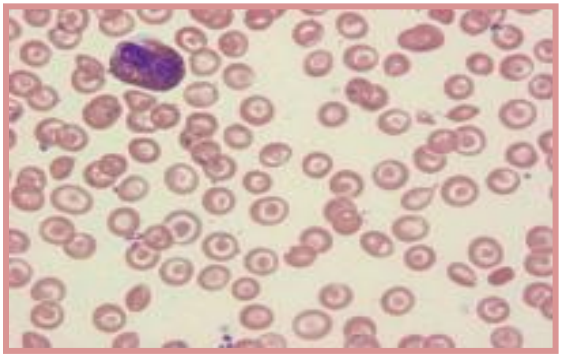

Figure 26: Blood film showing target cells.

Figure 27: Blood film showing leptocytes.

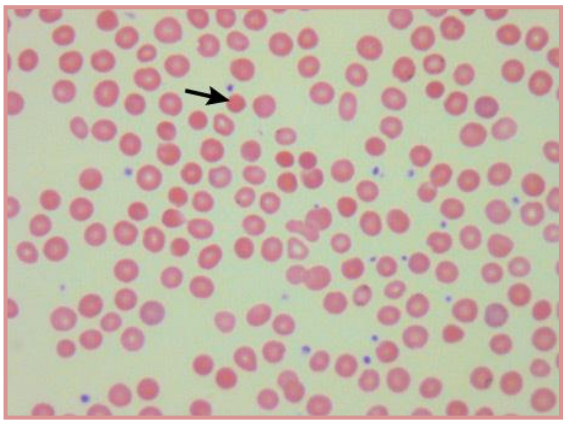

Figure 28: Blood film showing spherocyes.

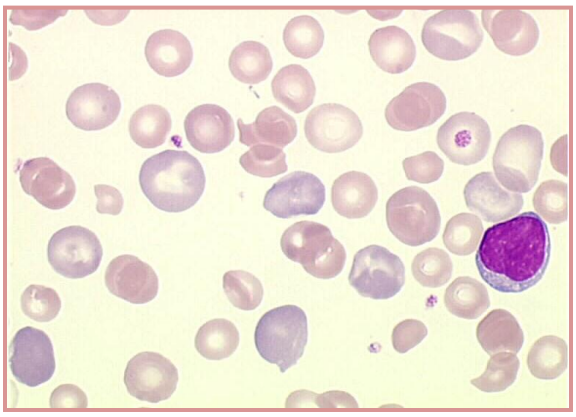

Figure 29: Blood film showing polychromasia, Blue coloration of RBCs, a sign of immaturity

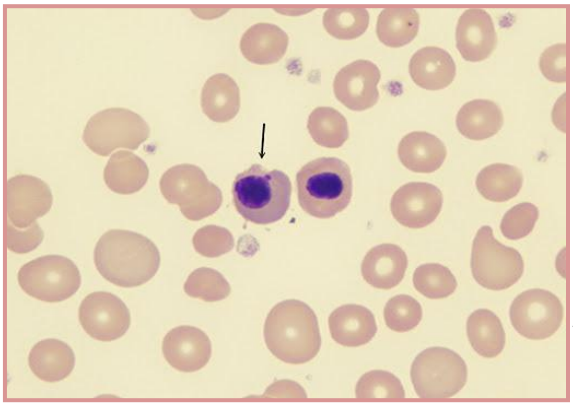

Figure 30: blood film showing nucleated "iimature"RBCs

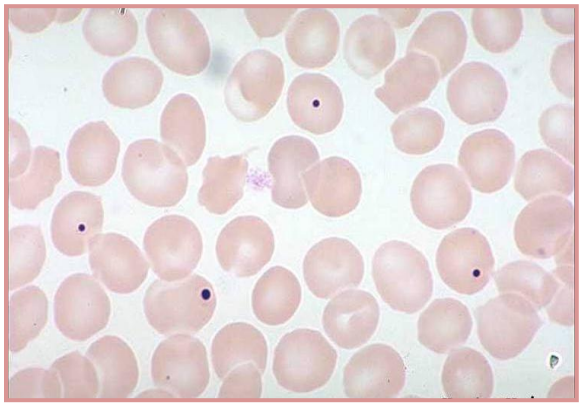

Figure 31: Blood film showing howell jolly bodies.

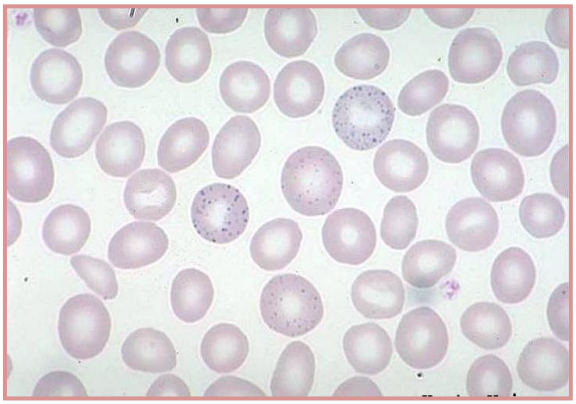

Figure 32: Blood film showing basophilic stippling.

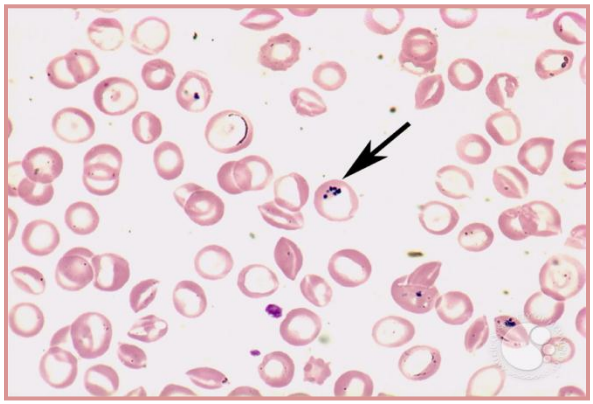

Figure 33: Blood film showing pappenheimer bodies.

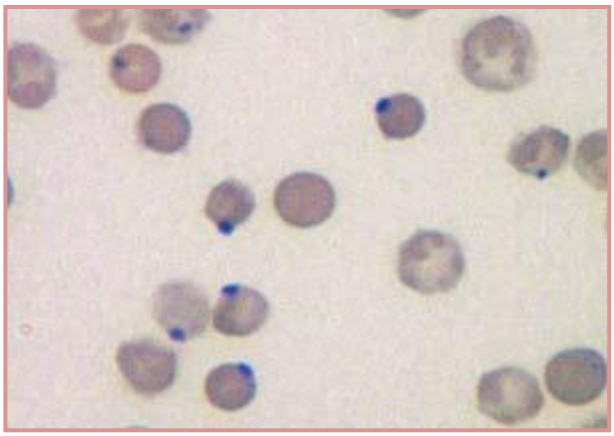

Figure 34: Blood film showing Heainz bodies.

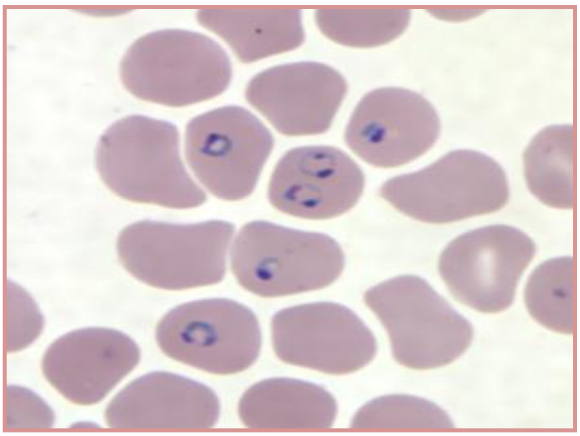

Figure 35: Blood film showing malarial parasite.

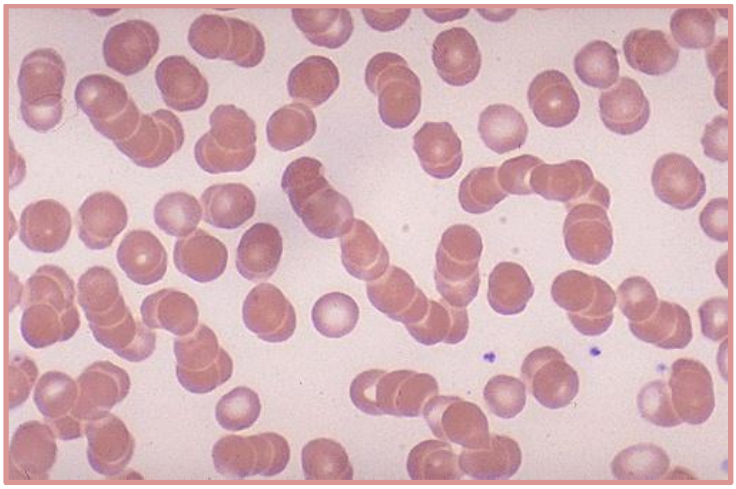

Figure 36: Rouleaux formation.

Erythrocyte Sedimentation Rate (ESR)

If anticoagulated blood is allowed to stand undisturbed in a vertical tube, the red cells will gradually settle to the bottom of the tube leaving a clear layer of plasma.

Red blood cells possess a negative charge and when suspended in normal plasma, rouleaux formation is minimal and the sedimentation of the cells is slow. Changes in the proportion and concentration of plasma proteins, particularly macro-molecules like fibrinogen and globulin, reduce the negative charge and thus increase the rate of rouleaux formation. This in turn increases the rate of sedimentation.

The ESR test is a non-specific test that indicates changes in plasma protein concentrations due to infection or injury.

Definition

The erythrocyte sedimentation rate (ESR) is the rate at which the red blood cells settle in a tube over a given amount of time.

Factors Affecting ESR

Plasma Protein

Changes in plasma protein occur rapidly following tissue injury or in response to inflammation. Increased concentration of fibrinogen and immunoglobulins will increase roulaux formation and hence the rate of sedimentation. Plasma albumin retards sedimentation of RBCs.

RBC Size and Number

The size and number of RBCs that show alterations in their biconcavity, such as spherocytes and sickle cells, usually do not increase the ESR rate, unless there is severe anemia. Increased cell mass will decrease the sedimentation rate (e.g. polycythemia).

Technical Factors

- Tilted ESR tube. ESR tubes should always be in a perpendicular (straight up) position. Slight deviations from the absolute vertical can increase results.
- A temperature higher than room temperature (18- 25 º C) accelerates sedimentation. Therefore, ESR tubes should not be exposed to direct sunlight or anything that may alter their temperature. If the test is to be carried out at a higher temperature, a normal range should be established for that temperature.
- Vibration can reduce the rate of erythrocyte sedimentation.

Methods of Measuring ESR

There are two methods for ESR measurement: The Westergren method and the Wintrobe method. The recommended method is the Westergren Method.

Westergren Method

Principle

The test measures the sedimentation of red cells (in diluted blood) after one hour in an open-ended tube mounted vertically on a stand. The recommended tube is a straight glass or rigid plastic transparent tube 30 cm in length and not less than 2.55 mm in diameter. The bore must be uniform to within 5% throughout (0.05 mm).

Procedure

1. Venous blood is collected into a sodium citrate tube, or collected into an EDTA tube and diluted accurately in a proportion of 1 volume of citrate to 4 volumes of blood. The usual practice is to collect blood directly into the sodium citrate tube.
2. Mix the blood sample thoroughly and then draw it up into the Westegren tube to the 200 mm mark by means of a mechanical suction device.
3. Place the tube exactly vertical and leave undisturbed for exactly 60 minutes, free from vibrations and not exposed to direct sunlight
4. After the 60 min are over, read to the nearest 1 mm the height of the clear plasma above the upper limit of the column of sedimenting cells.
5. The result is expressed as ESR= X mm in 1 h.

Notes on procedure:

- The test should be carried out within 4 hours of collecting the blood. A delay up to 6 hours is permissible provided that the blood is kept at 4◦C.
- EDTA blood can be used 24 hours on a specimen that was kept in 4◦C if 1 volume of 109 mmol/l trisodium citrate is added to 4 volumes of blood immediately before the test is peformed.

ESR Normal Ranges

Men

- Ages 17-70: 10 – 14 mm/hour
- Ages > 70: about 30 mm/hour

Women

- Ages 17-70: 12-20 mm/hour
- Ages >70: about 35 mm/hour

Pregnant Women:

- First half: 48 mm/hour (62 if anemic)
- Second half: 70 mm/hour (95 if anemic)

General Applications of ESR

Conditions that Cause a High ESR

- **Malignancy:**
  - Malignant lymphoma
  - Breast and colon carcinomas
- **Hematologic:**
  - Macrocytosis.
  - Anemia of acute or chronic diseases
- **Inflammatory Disorders:**
  - Rheumatoid arthritis
- **Infections:**
  - Tuberculosis
  - Acute hepatitis

Conditions that Cause a Low ESR

- Polycythemia
- Sickle cell anemia
- Hypofibrinogenemia
- Congestive Heart failure
- Leukocytosis

Note:
Other factors that influence ESR include: age, sex, menstrual cycle and the use of certain drugs.

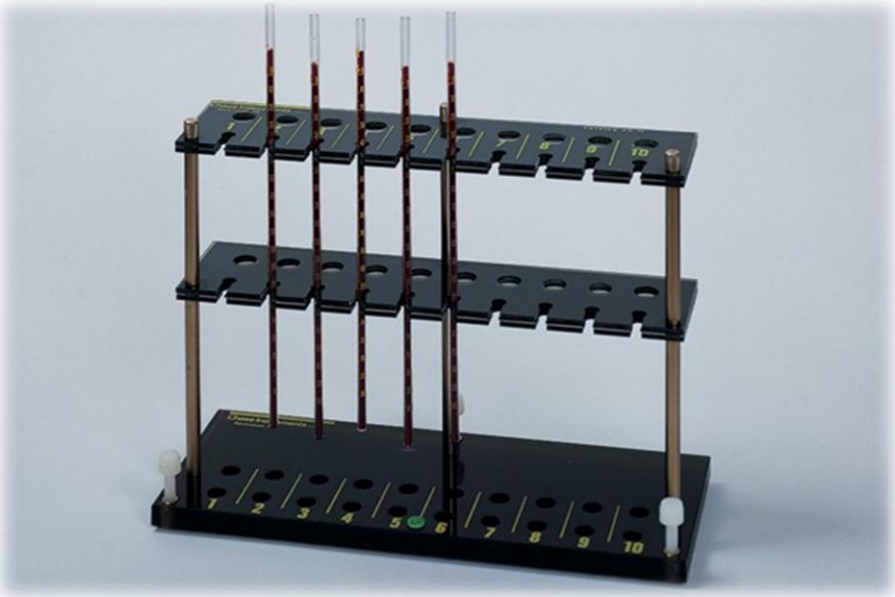

Figure 37: Westergren ESR tube rack

Anemias

Normal Blood Film

A normal blood film shows normally-shaped red blood cells which are normocytic and normochromic, normal number and shape of platelets and normal leukocyte shape and number.

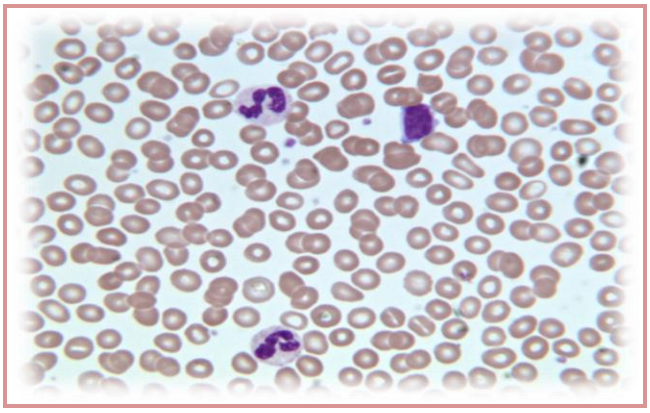

Figure 38: Normal blood film. Cells are normocytic and normochromic.

Iron Deficiency Anemia

Iron deficiency anemia occurs when the reticuloendothelial iron stores (hemosiderin and ferritin) become completely depleted.

Blood Smear

- Microcytic, hypo-chromic RBCs.

Laboratory Findings

- *General Tests:*
  - Hb ↓
  - PCV ↓
  - MCV↓, MCH ↓

- *Confirmatory Tests:*
  - Serum iron ↓
  - Serum ferritin ↓
  - Total iron binding capacity(TIBC) ↑
  - Serum transferrin receptor ↓
  - Bone marrow iron stores: absent
  - Erythroblast iron: absent

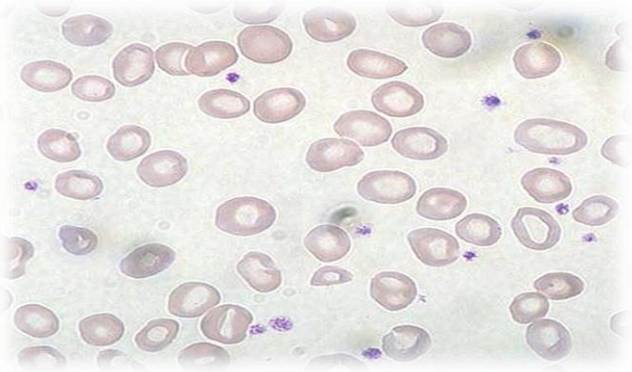

Figure 39: Iron deficiency anemia. The cells are hypochromic and microcytic.

Megaloblastic Anemia

This is a type of anemia where the erythroblasts in the bone marrow show a characteristic abnormality-maturation of the nucleus being delayed relative to that of the cytoplasm. The underlying defect accounting for the asynchronous maturation of the nucleus is defective DNA synthesis, which is usually caused by deficiency of vitamin B_{12} or folate.

Blood Smear

- Macrocytic anemia
- Hypersegmented neutrophils (>5 lobes)

Laboratory Findings

- *General tests:*
  - Hb ↓
  - PCV↓
  - MCV ↑ **(** > 95 fl)
  - WBC and platelets are reduced in severe anemia

- *Confirmatory Tests:*
  - Serum and red cell folate assay ↓
  - Serum vitamin B_{12} assay ↓
  - BM examination shows megaloblastic changes **(**Hyper cellular BM with large erythroblasts due to defective DNA synthesis + failure of nuclear maturation)

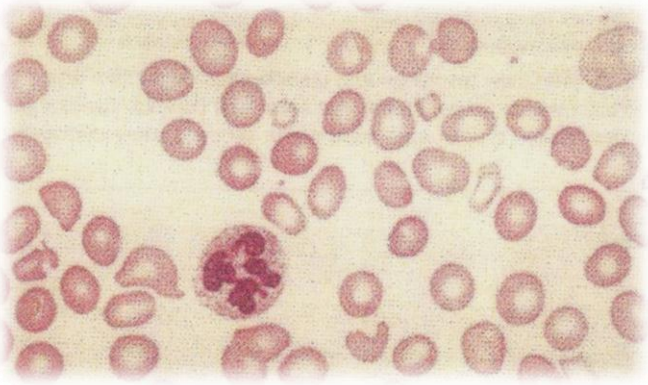

Figure 40: Megaloblastic anemia. The red cells are macrocytic.

Sickle Cell Anemia

Sickle cell disease is a group of hemoglobin disorders in which the sickle β-globin gene is inherited. The sickle β-globin abnormality is caused by substitution of valine for glumatic acid at position 6 in the β chain. Hb S is insoluble and forms crystals when exposed to low oxygen tension causing the red cell shape to change into a sickle shape.

Blood Smear

- Sickle cells and target cells
- Features of splenic atrophy (e.g. howell jolly bodies) may also be present

Laboratory Findings

- *General Tests:*
  - Hb ↓
- *Screening Tests:*
  - Solubility test
  - Sickling test
- *Confirmatory Test:*
  - Hb electrophoresis

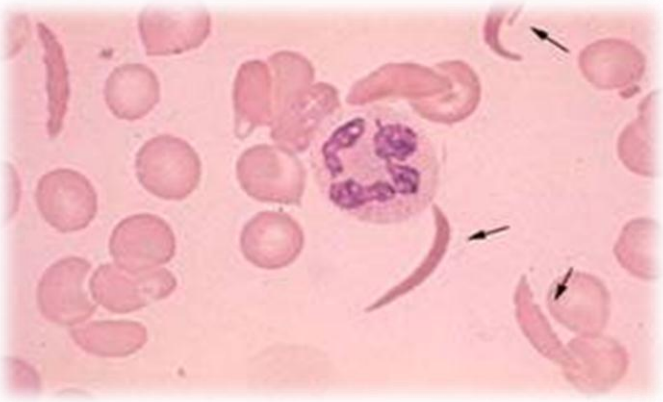

Figure 41: Sickle cell anemia: peripheral blood film showing sickle cells.

Hemolytic Anemias

Hemolytic anemias are classified into two categories: hereditary and acquired hemolytic anemia. Hereditary hemolytic anemias usually result from intrinsic red cell defects whereas acquired hemolytic anemias are usually the result of an extracorpuscular or environmental change. Refer to the table on the following page for the classification of hemolytic anemias.

Classification of Hemolytic Anemias

Hereditary	Acquired
Membrane defects Hereditary spherocytosis, hereditary elliptocytosis	**Immune** *1. Autoimmune* - Warm antibody type - Cold antibody type *2. Alloimmune* - Hemolytic transfusion reactions - hemolytic disease of the new born *3. Drug associated*
Defective red cell metabolism G6PD deficiency, pyruvate kinase deficiency	**Red cell fragmentation syndromes**
Defective hemoglobin synthesis	**March hemoglobinuria**
	Infections - Malaria - Clostridia
	Chemical and physical agents - drugs - industrial domestic substances - burns
	Secondary Liver and renal disease
	Paroxysmal nocturnal hemoglobinuria

Table 2: Classification of hemolytic anemias

Red Cell Membrane

The red blood cell membrane is a phospholipid bilayer composed of proteins 50%, lipids 40% and carbohydrates 10%. The red cell proteins are either integral or peripheral proteins. The membrane skeleton is formed by structural proteins that include:

- α and β spectrin
- ankyrin
- protein 4.1
- actin

Defects in these proteins lead to some abnormalities in the shape of the red cell membrane i.e. spherocytosis and elliptocytosis.

Hereditary Spherocytosis

Hereditary spherocytosis is caused by the following:

- Ankyrin deficiency or abnormalities
- Spectrin deficiency or abnormalities
- Protein 4.2 abnormalities

These defects lead to loss of the biconcave shape of red blood cells causing them to become spherical in shape.

Blood Smear

- Micro-spheroycytes

Laboratory findings

- Hb ↓, RBC ↓
- MCV ↓, MCHC ↑
- High or normal MCH
- Reticulocytes ↑ (due to increased bone marrow activity)
- The classic finding is increased osmotic fragility
- Direct antiglobulin test (coomb'c test) is –ve

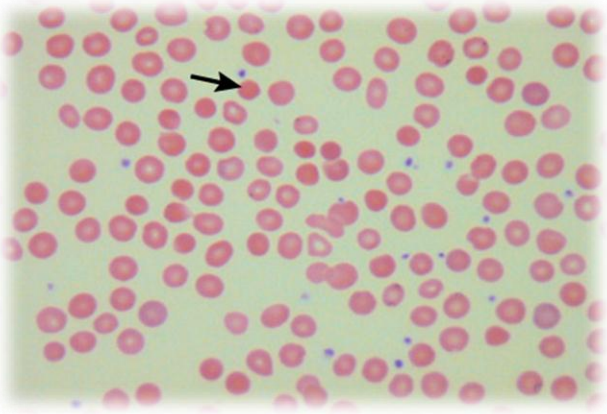

Figure 42: Hereditary spherocytosis. The red cells are spherical in shape with no central pallor

Hereditary Elliptocytosis

Hereditary elliptocytosis is caused by the following causes:

- α or β spectrin mutants leading to defective spectrin dimer formation
- α or β spectrin mutants leading to defective spectrin-ankyrin associations
- protein 4.1 deficiency or abnormality
- band 3 abnormality

Blood smear

- Elliptocytes

Laboratory findings

Laboratory findings are similar to those of hereditary spherocytosis.

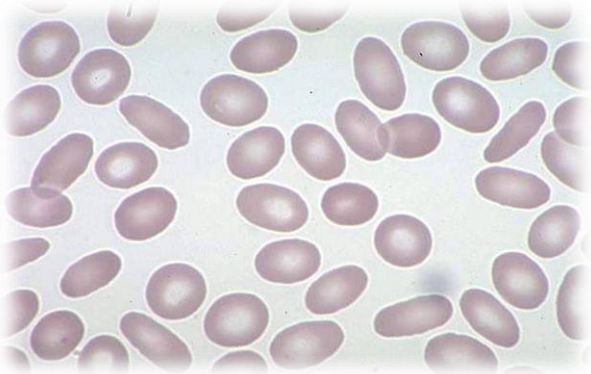

Figure 43: Hereditary elliptocytosis. The red cells are elliptical in shape.

Thalassemias

These are a heterogeneous group of genetic disorders which result from reduced rate of synthesis of α or β globin chains.

The globin genes are arranged on chromosomes 11 and 16 in the order in which they are expressed. There are 4 α-globin genes on chromosome 16 and 2 β-globin genes on chromosome 11.

α-Thalassemia Syndromes

These are usually caused by gene deletions. As there are normally 4 copies of the α-globin gene, the clinical severity depends on the number of genes that are missing or inactive.

Hydrops fetalis

Loss of all four α genes completely suppresses α-chain synthesis. Since the α-chain is essential in fetal as well as adult Hb, this is incompatible with life and leads to death in utero (hydrops fetalis). The hemoglobin that is present in affected fetuses is hemoglobin barts (γ_4)

Hemoglobin H disease

Three α-gene deletions lead to a moderately sever microcytic, hypochromic anemia with splenomegaly. This is known as Hb H disease because Hb H (β_4) can be detected in the red cells of these patients by Hb electrophoresis.

Blood smear:

- Microcytic, hypochromic cells
- Target cells and poikilocytosis
- Polychromasia

Laboratory findings:

- Hb ↓, RBC ↓
- MCV ↓,MCH↓ , MCHC↓
- Incubation of red blood cells with brilliant crysyl blue reveals the presence of inclusion bodies.
- Confirmatory test: Hb electrophoresis shows a pattern of Hb A, Hb A_2 and Hb barts.

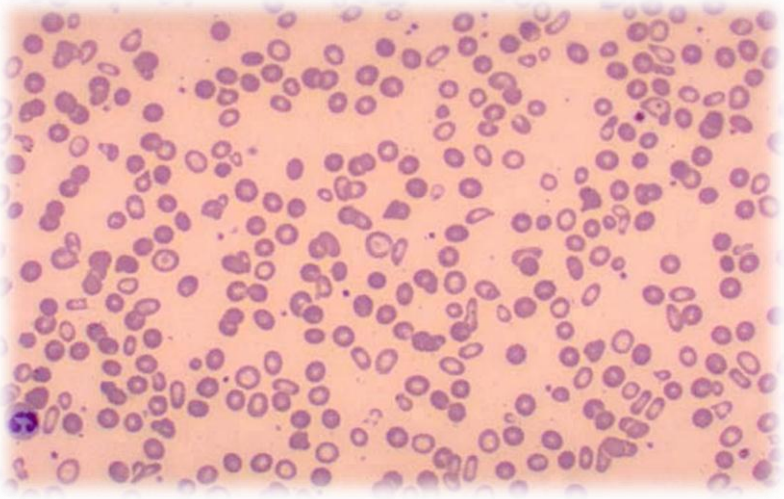

Figure 44: Hemoglobin H disease. Peripheral blood shows hypochromic, microcytic cells, target cells and bizarre shapes.

α-thalassemia traits

The α-thalassemia traits are caused by loss of one or two genes and are usually not associated with anemia. However, the MCV and MCH are low and the RBCs are slightly high. Hb electrophoresis is normal.

β-Thalassemia Syndromes

These are characterized by reduced or completely absent β-globin chains mainly due to point mutations.

β- thalassemia major

Either no β-chains are synthesized (β^0), or small amounts of β-chains are synthesized (β^+). Excess α-chains precipitate in erythroblasts and mature RBCs causing severe ineffective erythropoiesis and hemolysis. The greater the α-chain excess, the more severe the anemia.

Blood smear:

- Hypochromic, microcytic RBCs
- Basophilic stippling
- Target cells
- Normoblasts (nucleated RBCs)

Laboratory findings:

- Hb↓
- MCV↓
- MCH ↓
- Confirmatory test: Hb electrophoresis shows absence or almost complete absence of Hb A with almost all the circulating hemoglobin being Hb F.

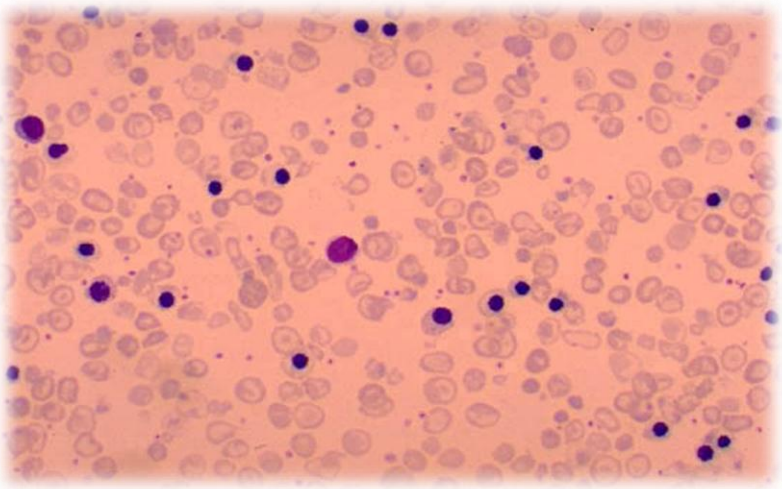

Figure 45: β-thalassemia major. Peripheral blood film of severe beta thalassemia shows microcytic, hypochromic cells, targert cells and nucleated RBCs.

β- thalassemia trait (minor)

This is a common usually symptomless abnormality characterized by a hypochromic microcytic blood picture, but high in RBC count. It is usually more severe than α-thalassemia trait.

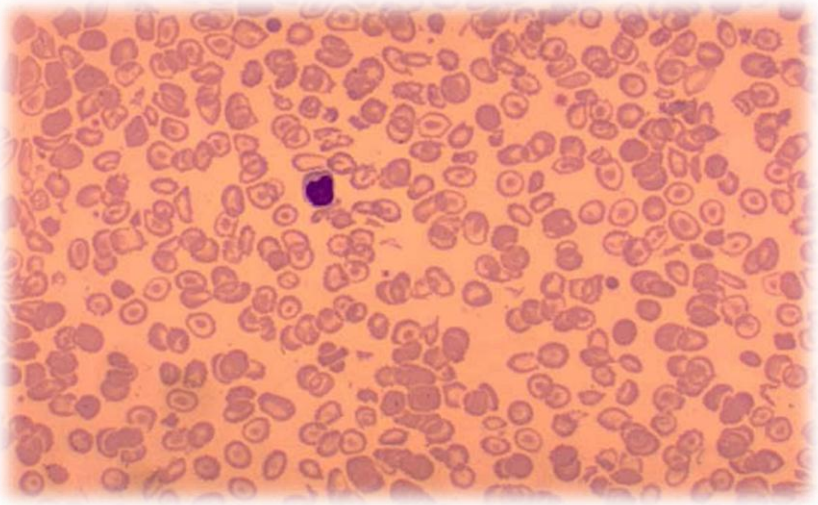

Figure 46: β-thalassemia minor. Peripheral blood film shows microcytic, hypochromic and target cells.

Tests for Abnormal Hemoglobin S

Hemoglobin S is the most common structural variant of Hb. As a result of the replacement of glutamic acid by valine position 6 of the β chain, Hb S has poor solubility in the deoxygenated state and can polymerize in the red cells. The red cell shows a characteristic shape change because of polymer formation and becomes distorted and rigid, the co-called sickle cell.

Forms of Sickle Cell Disease

Hb SS

The homozygous state or sickle cell anemia (genotype SS) causes moderate to severe hemolytic anemia. The main clinical disability arises from repeated episodes of vascular occlusion by sickled RBCs resulting in acute crises and eventually in end-organ damage. The clinical severity of sickle cell anemia is extremely variable. This is due to the effect of inherited modifying factors (e.g.: interaction with β thalassemia), and socioeconomic conditions or other factors that influence general health.

Note:
Hb SS is composed of two α chains and two βS chains.

Hb AS

The heterozygous state or sickle cell trait (genotype AS) is very common and it affects millions of people worldwide. It is known as the carrier state. There are no associated hematological abnormalities. In vivo sickling occurs only at very high altitudes and low oxygen pressures.

Note:
Hb AS is composed of two α chains, one β chain and one βS chain.

Other Forms of Sickle Cell Disease

- *Sickle cell/ Hb C disease*

  This is a compound heterozygous state for Hbs S and C. It usually results in a milder form of sickle cell disease.

- *Sickle β/thalassemia*

  This disease arises as a result of inheritance of one Hb S gene and one β thalassemia gene.

Screening Tests for Sickle Cell Disease

These tests depend on the decreased solubility of Hb S at low oxygen tensions. All sickle tests, whether positive or negative, must be confirmed by electrophoresis or HPLC at the earliest opportunity.

Sickling Test

Principle

The sickling phenomenon may be demonstrated in a thin wet film of blood sealed with petroleum jelly/paraffin wax mixture or nail varnish. If Hb S is present the red cells lose their smooth round shape and become sickled. Changes should be apparent after 30 min- 1 hour at 37◦ C.

Reagent

The reagent is 2% sodium metabisulphite. It should be freshly prepared.

Procedure

1. On a clean slide, add 1 drop of anticoagulated blood.
2. Add 5 drops of the freshly prepared reagent to the drop of blood.
3. Mix and place a cover slip on top of the slide.
4. Place the slide inside a wet petri-dish and seal with a paraffin wax/ petroleum jelly mixture or nail varnish.
5. Incubate for 30 min at 37◦ C.
6. Examine under the microscope (x40)

Results

- **+ve:** A positive result is indicated by the presence of sickled cells. Hb SS, Hb AS, Hb S/C and Hb S/β thalassemia all yield +ve results.
- **-ve:** a negative result means there are no sickle cells and the cells are round in shape. Hb AA yields a –ve result.

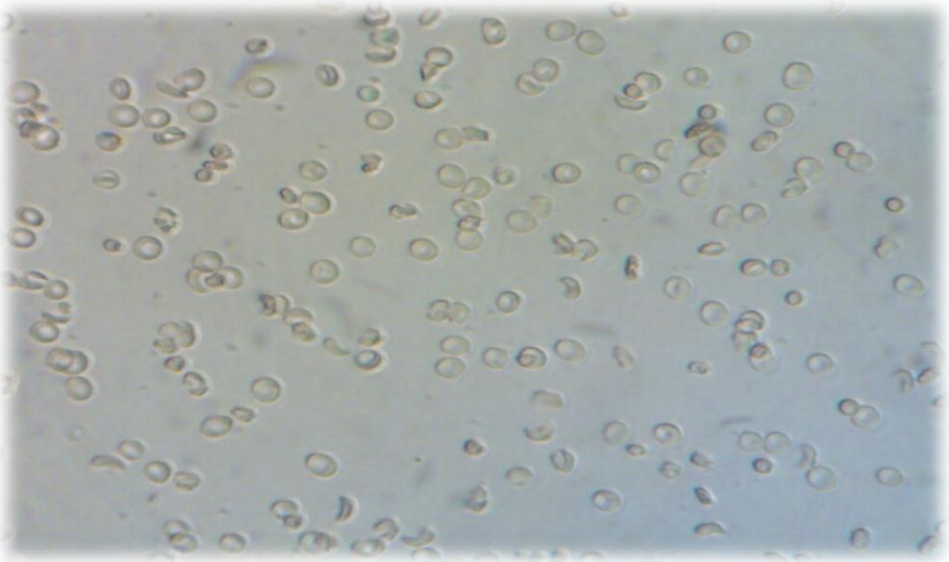

Figure 47: positive sickling test.

Solubility Test

Principle

Sickle cell Hb is insoluble in the deoxygenated state in a high molarity phosphate buffer. The crystals that form refract light and cause the solution to be turbid. Cells containing Hb S resist lysis, which causes the solution to become turbid due to the insolubility of Hb S. Cells containing normal Hb, are prone to lysis, and thus the Hb is dissolved giving a clear solution.

Reagents

Sodium dithionite and a lysing agent.

Procedure

1. Pipette 4 ml of buffer into three solubility test tubes containing sodium dithionite (the tubes are from the testing kit and they already contain sodium dithionite).
2. Mix the contents of the tube.
3. Add 50 micro liters of EDTA packed cells.
4. Mix well and leave to stand for 10-15 minutes.
5. Place the tube on a white rack with narrow black lines and read for turbidity (see if the lines are clearly visible or not).
6. Record the results.

Results

- a positive result is indicated by a turbid solution through which the black lines could not be seen.
- a negative result is indicated by a clear solution through which the lines could be easily seen.

Notes:

- False +ve results have been reported in sever leukocytosis, in hyperproteinemia and in the presence of an unstable Hb. The use of packed cells minimizes the problem of false +ves caused by hyperproteinemia.
- False –ve results can occur in patients with a low Hb, and the use of packed cells will overcome this problem. False –ves may also occur if expired reagents are used.

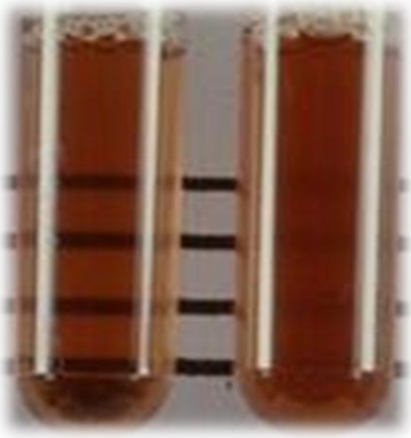

Figure 48: Solubility test for sickle cell anemia. The tube on the left shows a –ve result (clear) whereas the tube on the right represents a +ve result (turbid) .

Reticulocyte Count

Reticulocytes are immature red blood cells which contain remnants of ribosomal RNA. They are normally present in the bone marrow and blood stream. The number of reticulocytes in peripheral blood is a reflection of erythropoietic activity.

Principle

Given their acidic nature, ribosomes of reticulocytes react with certain alkaline dyes such as brilliant cresyl blue or new methylene blue to form dark blue/purple granules or filaments. This reaction takes place only in vitally-stained unfixed preparations. If, however, blood films are dried, fixed and then stained with basophilic dyes, reticulocytes appear as diffused basophilic RBCs (polychromatic RBCs). Since reticulocytes ripen within about 24 hours in blood circulation, the reticulocyte count must be done on fresh blood.

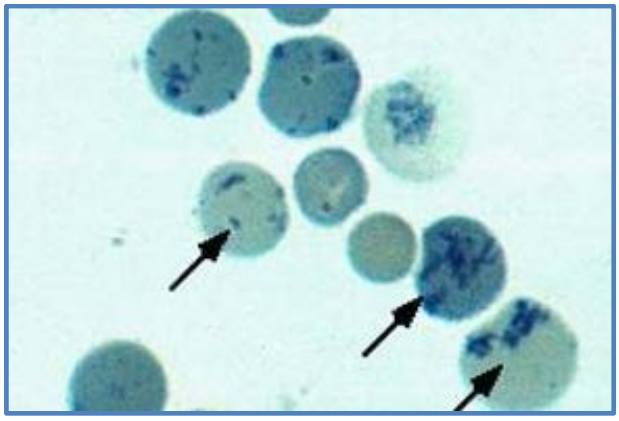

Figure 49: Reticulocytes in peripheral blood. They appear as greenish cells with blue filaments.

Method

1. Add 2-3 drops of reticulocyte dye in a tube.
2. Add 2 drops of blood and mix.
3. Incubate at 37°C for 15-20 min.
4. Re-suspend by gentle mixing and prepare a film on s glass slide. Leave to dry.
5. Examine under microscope using oil immersion lens.
6. Select a well prepared, well stained area. Reticulocytes appear as pale greenish cells with dark blue granules or filaments.
7. Count the number of reticulocytes in 40 successive fields.
8. Count the number of total RBCs in the fields: 1, 10, 20 and 30.
9. Determine the % ratio of reticulocytes to total RBCs.

Calculation

Number of reticulocytes in 40 fields = X

Number of total RBCs in four fields = Y

Reticulocyte count = $\frac{X}{N \times Y} \times 100\%$

Normal Ranges

- Adults: 0.5 -2.5 %
- Infants: 2-5 %

Abnormal Reticulocyte Count

Reticulocytosis

Reticulocytosis is a high reticulocyte count. It occurs in the following conditions:

- Rapid blood loss
- Ineffective erythropoiesis e.g. thalassemia.
- Malignant disease.
- Lack of erythropoietin
- Hemolytic anemias e.g. hereditary elliptocytosis, hereditary spherocytosis.

Low reticulocyte count

Low reticulocyte count occurs in the following conditions:

- Aplastic anemia
- Iron deficiency
- Exposure to radiation
- Long term chronic infection
- Certain medications that damage the bone marrow .

Automated Reticulocyte Count

Automated reticulocyte counts have been developed by using the fact that various dyes and fluorochromes combine with the RNA of reticulocytes. Following binding of the dye, fluorescent cells can be enumerated using a flow cytometer. Most fully automated blood counters now incorporate a reticulocyte counting capacity.

Hemoglobin Electrophoresis

Electrophoresis is a process by which molecules can be separated according to their molecular weight and electrical charge by applying electric current to them. Each molecule travels through the medium at a different rate depending on its size and charge, and on the type of medium used. There are different types of media for electrophoresis e.g. agarose gel, acrylamide gel, cellulose acetate and paper.

Hemoglobin Electrophoresis

Hemoglobin electrophoresis at pH 8.4-8.6 using cellulose acetate membrane is a rapid, simple, reliable confirmatory test. It is satisfactory for the detection of the most common clinically important hemoglobin variants.

Principle

At alkaline pH, hemoglobin is a negatively charged protein and when subjected to electrophoresis will migrate toward the anode (+). Structural variants have a change in the charge on the surface of their molecule. This will cause them to separate from Hb A at alkaline pH. Migration of molecules depends on their molecular weight and electrical charge. The higher the negative charge the faster the movement.

Notes:
- Hb A has the highest amount of negative charges, and thus it is the fastest in migration.
- Hb A is soluble, has a low molecular weight and has a high negative charge.
- Hb S is insoluble, has a low molecular weight and a lower negative charge.

Equipment

- Electrophoresis tank and power source
- Wicks of filter
- Blotting paper
- Applicators
- Cellulose acetate membrane.

Reagents

- Electrophoresis buffer: tris EDTA/ borate (TEB) pH 8.5
- Wetting agent
- Ponceau stain
- Destaining solution: 5% acetic acid
- Hemolysing agent

Method

1. Centrifuge samples at 1200g for 5 min. Dilute 20 µl of packed cells with 150 µl of the hemolyzing agent. Mix gently and leave for 5 min. if purified hemolysates are used, 40 µl of 10 g/dl hemolysate with the 150 µl of lysing agent.

2. With the power supply disconnected, prepare the electrophoresis tank by placing equal amounts of TEB buffer in each of the outer buffer compartments. Wet 2 chamber wicks in the buffer, and place one along each divider/bridge support ensuring that they make good contact with the buffer.
3. Soak the cellulose acetate by lowering it slowly into a reservoir of buffer; leave the cellulose acetate to soak for 5 min before use.
4. Fill the sample well plate with 5 µl of each diluted sample/control and cover with coverslip or a short glad slide to prevent evaporation. Load a second sample well plate with the wetting agent.
5. Clean the applicator tips immediately prior to use.
6. Remove the cellulose strip from the buffer and blot twice between two layers of blotting paper.
7. Load the applicator by depressing the tips into the sample wells twice, and dispense this first load. Reload the applicator and apply samples into cellulose acetate.
8. Place the cellulose acetate plates across the bridges, with the plastic side uppermost. Place two glass slides across the strip to maintain good contact. Electrophorese at 350 V for 25 min.
9. After 25 min electrophoresis, immediately transfer the cellulose acetate to ponceau S and fix and stain for 5 min.
10. Remove excess stain by washing for 5 min in the 1st acetic acid reservoir and for 10 min in each of the remaining two. Blot once, using blotting paper and leave to dry.
11. Label the membranes and store in protective plastic envelope.

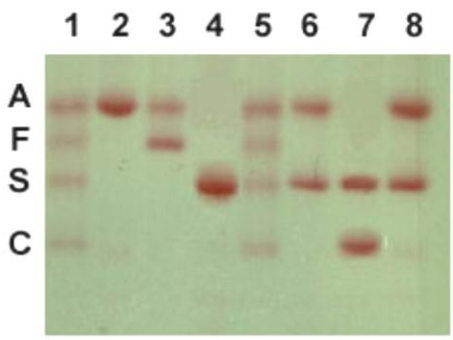

Figure 50: pattern of hemoglobin electrophoresis from several patients. Lanes 1 and 5 are Hb standards. Lane 2 is a normal adult. Lane 3 is a normal neonate. Lane 4 is a homozygous HbS. Lanes 6 and 8 are heterozygous sickle individuals. Lane 7 is a SC disease.

Automated Hb Electrophoresis

In today's modern laboratories, there are automated electrophoresis machines which offer a new level of continuous processing electrophoresis automation. With true walk-away convenience, the system requires technologist intervention only to load samples, reagents, and slides, and to start the run. The technologist reviews and prints results when processing is complete. No instrument monitoring is necessary and the technologist does not have to physically move gels around. These instruments perform all electrophoretic phases, including sample application, migration, staining, destaining, clearing, drying, and scanning in a fully automated process.

Total White Blood Cell Count

White cell count is an important component of the blood count. White cells can be counted either manually or automatically. Anticoagulated venous blood is added to a diluent at a specific volume. The diluent lyses the erythrocytes, but preserves leukocytes. The diluted blood is added to the hematocytometer chamber. The diluent used is 2% acetic acid +gentian violet.

Manual Method

1. Pipette 0.95 ml of the diluents in a 75x10 mm tube.
2. Add 0.05 ml (50 µl) of blood to the tube.
3. Tightly seal the tube and mix the contents for one min.
4. Fill the counting chamber by means of a capillary tube.
5. Count the cells in the four "white cell" corner squares.

Note:
Each one of the four corner squares "W" has an area of 1mm^2. These large squares contain 16 smaller secondary squares, each with an area of 0.04 mm^2

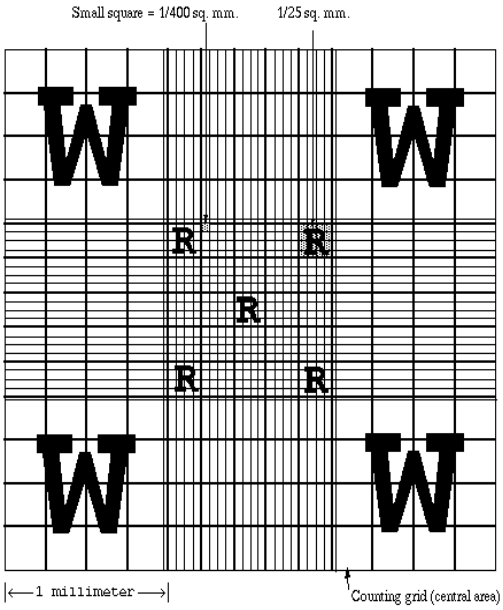

Figure 51: Hematocytometer. The areas indicated by "W" are used for counting white blood cells.

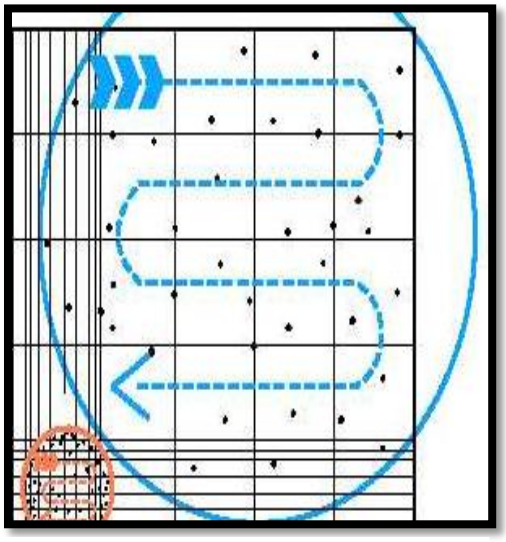

Figure 52: Illustration demonstrating the direction of counting WBCs on

Calculation

Dilution

$$\frac{50\ \mu l\ \text{blood}}{50} = 1\ \text{volume of blood}$$

$$\frac{0.95\ ml\ (950\ \mu l\ diluent)}{50} = 19$$

$$\frac{1}{19+1} = \frac{1}{20}$$

Dilution factor = 20

Number of WBC in Whole Blood

$$\text{Count} = \frac{\text{No.of cells counted}}{\text{volume}} \times dil\ factor$$

$$= \frac{N}{0.1} \times \text{dilution factor}$$

$$= N \times 10 \times 20 = N \times 200$$

Normal Range

The normal range is: $4 – 10 \times 10^9/l$

Automated White Blood Cell Count

Automated instruments are available to determine the WBC count. The total WBC is determined in whole blood in which red cells have been lysed. The lytic agent is required to destroy the red cells and reduce the red cell stroma to a residue that causes no detectable response in the counting system without affecting leukocytes in such a manner that the ability of the system to count them is altered. Various manufacturers require specific reagents, and for multichannel instruments that also perform an automated differential count use of the recommended reagent is essential.

White Blood Cell differential Count and WBC Morphology

In a normal blood film up to 5 different types of normal WBCs can be seen. They are neutrophils, lymphocytes, monocytes, eosinophils and basophils.The biological functions of WBCs include: defense and protection against microbial infections or any foreign substances, and the production of immunoglobulins.

Differential Count

In disease, either the normal percentage of WBCs is disturbed or/and abnormal or immature WBCs are seen in peripheral blood. The purpose of the WBC differential count is to determine the percentage of each type of WBC from a stained blood film.

Procedure

1. Place a drop of oil on a dry well stained blood film and place a clean cover slip on top.
2. Using the x10 objective, select an area which is well stained and well spread.
3. Switch power to the x40 objective lens.
4. Set the differential counter to zero.
5. Start counting all the WBCs seen in a single longitudinal direction pressing on the button of the differential counter that corresponds to the type of cell.
6. If the end of the selected area is reached before counting 100 cells, move one field up (or down). Then count in another longitudinal area going to the opposite direction.
7. Keep on counting in that manner until the alarm of the counter is heard. The total number of WBC counted should be 100.
8. Record the percentage of each WBC type directly from the counter

WBC Morphology

White blood cells are divided into two categories according to function: phagocytes and immunocytes. Phagocytes include granulocytes (neutrophils, basophils, eosinophils) and agranulocytes. Immunocytes include lymphocytes.

Neutrophils

In normal adults, neutrophils account for more than half the circulating leukocytes. They are the main defense of the body against pyogenic (bacterial) infections. Normal neutrophils are uniform in size (about 3 times larger than RBCs), with a diameter of about 13 μm on film. They have a segmented nucleus which stains deep violet, and a pink/orange cytoplasm with fine granulation**.**
The majority of neutrophils have three nuclear lobes connected by chromatic strands. A small percentage has four lobes, and occasionally five lobes are seen. Up to 8% of circulating neutrophils are unsegmented or partly segmented (band forms). In megaloblastic anemias, neutrophils become hypersegmented with five or more nuclear lobes. Hyper segmented neutrophils may also be seen in uraemia and in other conditions. Toxic granulation is the term used to describe an increase in

staining, density and number of granules that occurs regularly in bacterial infection and often with other causes of inflammation. The normal range of neutrophils in health is 40-80% (2-7 × 10^9/l).

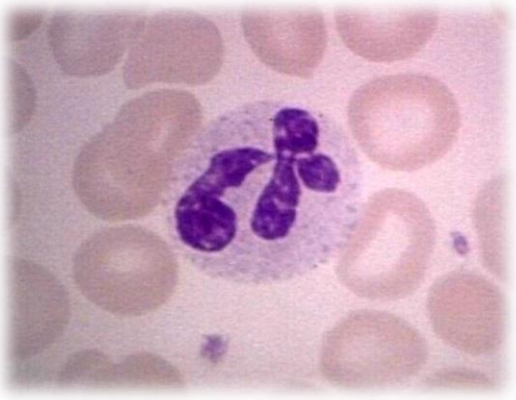

Figure 53: mature neutrophil usually having (3-5) lobes.

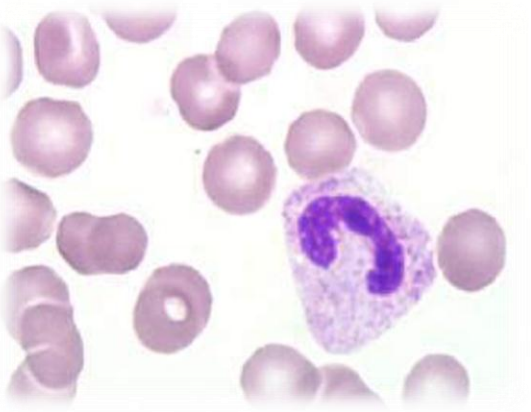

Figure 54: Band neutrophil

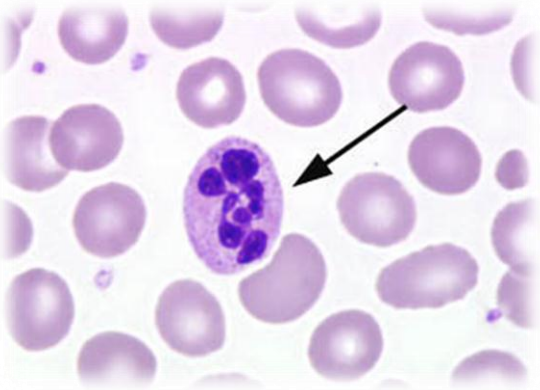

Figure 55: Hyper-segmented neutrophil

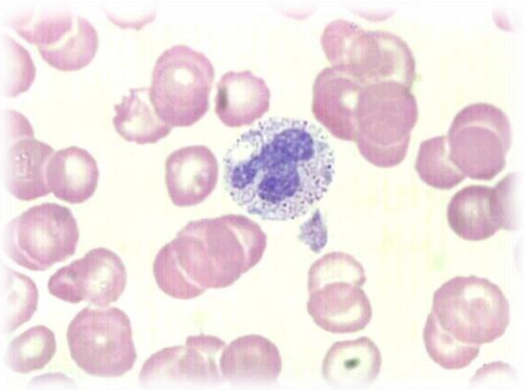

Figure 56: Toxic granulation

Eosinophils

Eosinophils are larger than neutrophils having a diameter of 12-17 µm. They contain 2 lobes in their nucleus that often appear like sunglasses or a head set. The underlying cytoplasm is packed with distinctive spherical red/orange (eosinophilic) granules. Prolonged steroid administration causes eosinopenia. Moderate eosinophilia occurs in allergic conditions and more severe eosinophilia may be seen in parasitic infections. The normal range for eosinophils is 1-6% (0.02-0.5 × 10^9/l).

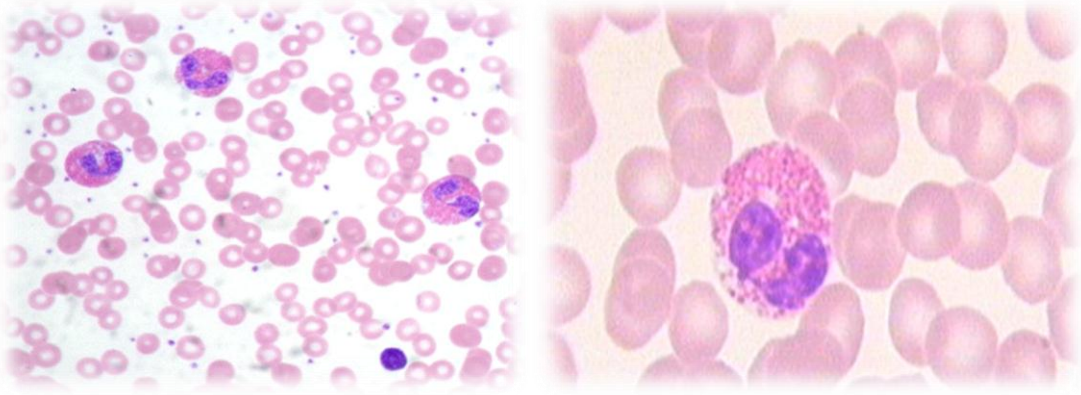

Figure 57: Eosinophils in Peripheral Blood Film. The nuclear lobes are shaped like sunglasses and the cytoplasm shows orange granules.

Basophils

They are the rarest of the circulating leukocytes and are only seen occasionally. Their nuclear segments tend to fold up on each other, resulting in a compact irregular dense nucleus resembling a closed lotus flower. The distinctive, large, variably sized, dark blue/purple granules of the cytoplasm often obscure the nucleus; they are rich in histamine, serotonin and heparin substances. Basophils tend to degranulate, leaving cytoplasmic vacuoles. The normal range of basophils is less than 1-2% (0.02-0.1× 10^9/l).

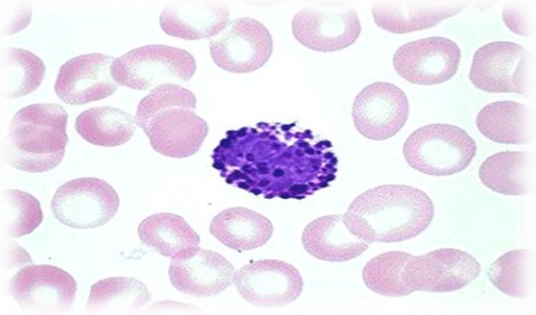

Figure 58: Basophil in peripheral blood.

Monocytes

Monocytes are the largest of the circulating WBCs, 15-18 µm in diameter. They have bluish/grey cytoplasm that contains variable numbers of fine reddish granules. The nucleus is large and curved, often in the shape of a horseshoe, but it may be folded or curled. It never undergoes segmentation. The chromatin is finer and more evenly distributed in the nucleus than in neutrophil nuclei.Vacuoles are sometimes seen in the cytoplasm. The functions of monocytes include: phagocytosis, interferon activation, chemotaxis and antigen presentation. The normal range of monocytes is 2-10% (0.1-1× 10^9/l).

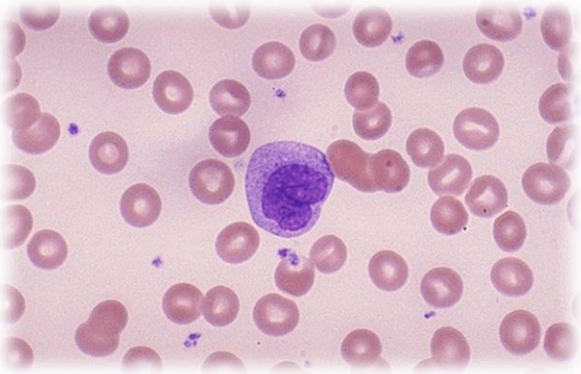

Figure 59: Monocyte. Monocytes are large WBCs with an irregular outline and a large nucleus. Cytoplasm is transparent and may contain vacuoles

Lymphocytes

Two unique features characteristic of the immune system are the phenomenon of immunological memory and the ability to generate antigen specificity. The immune response depends upon two types of lymphocytes: T-cells (helper and cytotoxic) and B- cells. A third type of lymphocytes is the natural killer cell. It is a large cytotoxic cell with cytoplasmic granules. B-lymphocytes are responsible for the production of immunoglobulins.

The majority of circulating lymphocytes are small cells with a thin rim of cytoplasm due to the large size of the nucleus. Nuclei are remarkably uniform in size staining deep purple and the cytoplasm is blue in color. A small amount of the lymphocytes are larger in size, with more abundant pale blue cytoplasm containing azurophilic granules. Their nucleus is less condensed than that of small lymphocytes and is round in shape. The normal range of lymphocytes is 20-40% (1-3 $\times$ 10^9/l).

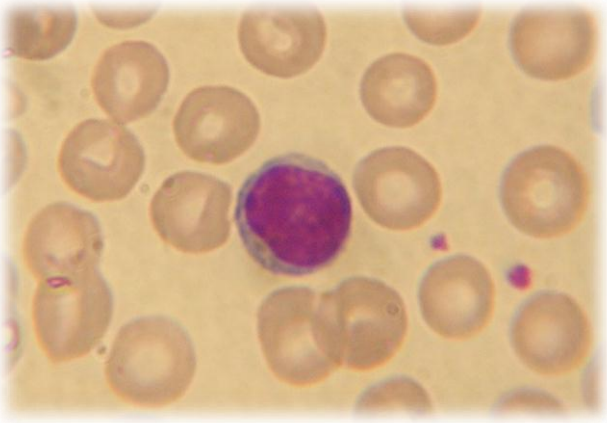

Figure 60: Small lymphocyte. The small lymphocyte has a thin rim of cytoplasm and a relatively large nucleus.

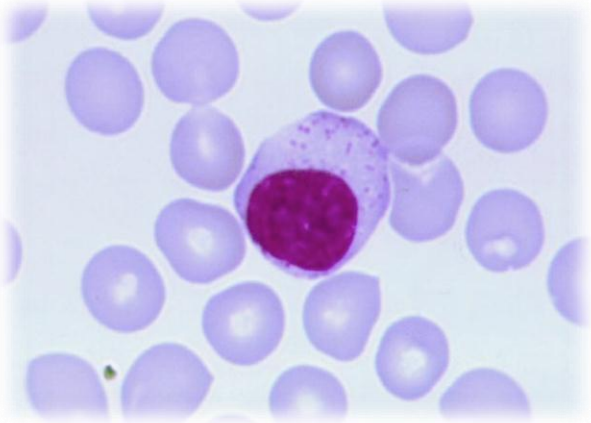

Figure 61: Large lymphocyte. This lymphocyte is larger than the other type. It has more abundant cytoplasm.

Automated Differential Count

Most automated differential counters that are now available use flow cytometry incorporated into a full blood counter rather than being stand-alone differential counters. Increasingly, automated blood cell counters have a differential counting capacity, providing either a three-part or a five- to seven-part differential count. Counts are performed on diluted whole blood in which red cells are either lysed or rendered transparent.

Quantitative Abnormalities of WBCs

Leukocytosis

Leukocytosis is an increase in the number of white blood cells. It is caused by:

- Chronic infections
- Inflammation
- Leukemia
- Allergy.

Leukopenia

Leucopenia is a decreased white blood cell count. It is caused by:

- Chemotherapy
- Radiation therapy
- Some types of cancer
- Malaria
- Tubercolosis

Neutrophilia

Neutrophilia is an abnormal increase in the number of neutrophils. Some of its causes are:

- Acute bacterial infection,
- Chronic granulocytic leukemia
- Iflammation
- Corticosteroid therapy

Eosinophilia

Eosinophilia is an abnormal increase in the number of eosinophils. It is caused by:

- Allergies
- Parasitic infections
- Drug sensitivity
- Skin diseases.

Basophilia

An abnormal increase in the number of basophils is called basophilia. It occurs during:

- Chronic granulocytic leukemia
- Delayed hypersensitivity reaction
- Hypothyroidism
- Nephrosis
- Ulcerative colitis.

Lymphocytosis

An abnormal increase in the number of lymphocytes. It is caused by:

- Viral infections (infectious mononucleosis, hepatitis, cytomegalovirus)
- Lymphoproliferative disorders (chronic lymphocytic leukemia, lymphoma)

Monocytosis

Abnormal increase in the number of monocytes. It is caused by:

- Chronic myelocytic leukemia
- Parasitic infections
- T.B.
- Subacute bacterial endocarditis
- Syphilis

Introduction to Haemostasis

An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is essential for survival. Such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The haemostatis system thus represents a balance between procoagulant and anticoagulant mechanisms allied to a process for fibrinolysis.

The haemostatic mechanisms have many important functions: (a) to maintain blood in a fluid state while it remains circulating within the blood vessels; (b) to stop bleeding at the site of injury by formation of a haemostatic plug; (c) to ensure the removal of the plug when healing is complete.

The five major components involved in haemostasis are, blood vessels, platelets, coagulation factors, coagulation inhibitors andfibrinolysis.

Blood Vessels

General Structure of the Blood Vessel

The blood vessel wall has three layers: intima, media and adventitia. The intima consists of endothelium and subendothelial connective tissue and is separated from the media by the elastic lamina interna. Endothelial cells form a continuous monolayer lining all blood vessels.

Endothelial Cell Function

The endothelial cell plays an important role in maintaining vascular integrity. Blood loss or damage to the endothelial lining results in both haemorrhage and activation of the haemostatic mechanism. Synthesis of tissue factor which initiates haemostasis only occurs in endothelial cells. The endothelial cell also has a strong inhibitory influence on the haemostatic response through the synthesis of certain vasodilators and the inhibition of platelet aggregation.

Vasoconstriction

Vessels contract following injury to stop blood loss. Although not all coagulation reactions are enhanced by reduced flow, this probably assists in the formation of a stable fibrin plug.

Platelets

Platelets are small fragments of cytoplasm derived from megakaryocytes. On average they are 1.5-3.5 μm in diameter. They do not contain a nucleus and are bounded by a lipid bilayer membrane. Platelet functions include: (a) adhesion to injured blood vessels; (b) secretion of platelet granule contents e.g. fibrinogen, heparin, serotonin…etc; (c) platelet aggregation at the site of vascular injury to plug the injured area; (d) platelet procoagulant activity.

Coagulation Factors

Blood coagulation involves a biological amplification system in which a cascade of precursor proteins (clotting factors) is activated by proteolysis. This cascade leads to the formation of thrombin, which converts plasma fibrinogen into fibrin. Fibrin enmeshes the platelet aggregates at the site of vascular injury and converts the unstable platelet plugs to firm stable haemostatic plugs. The coagulation cascade comprises two pathways: the intrinsic pathway and the extrinsic pathway. Refer to the scheme below (figure 61) for more details on the coagulation pathways. Some of the coagulation factors are vitamin K-dependent. These are factors II, VII, IX and X).

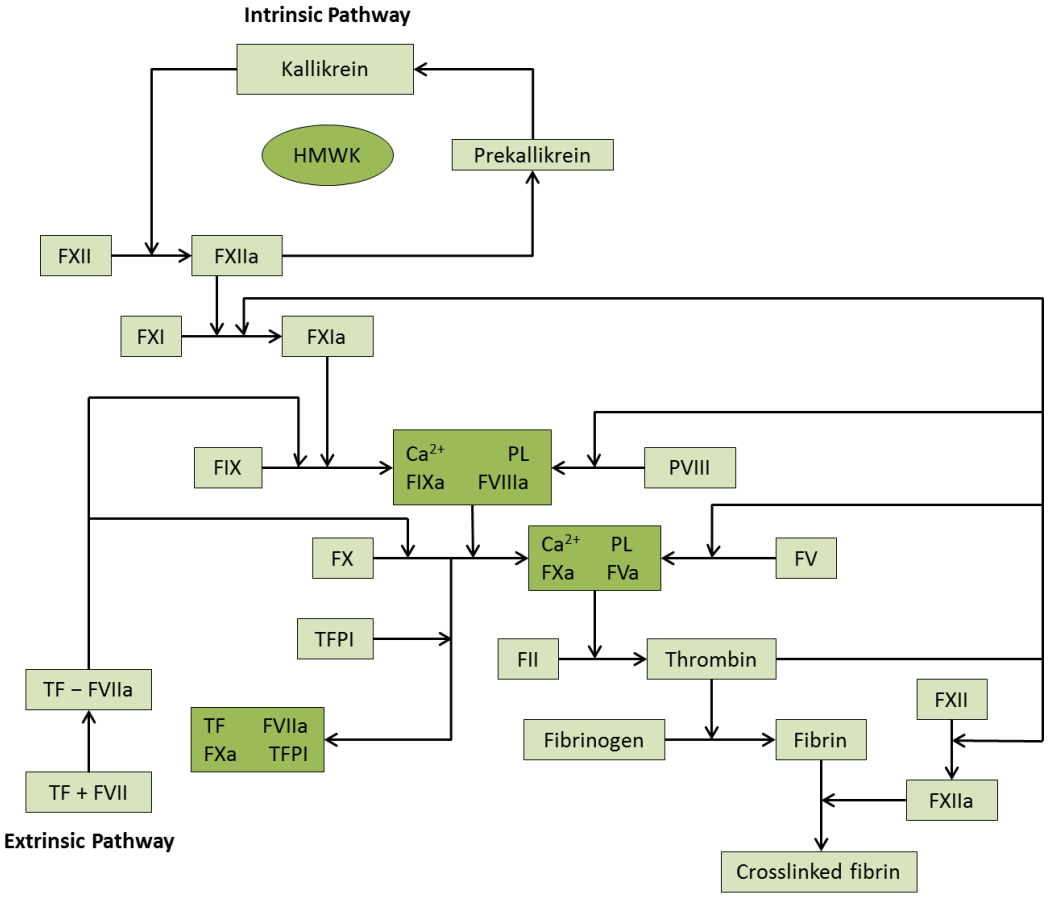

Figure 62: a scheme illustrating the two pathways of the coagulation system

Coagulation Inhibitors

It is important that the effect of thrombin is limited to the site of injury. This is achieved by the inhibition or inactivation of clotting factors by coagulation inhibitors.

Fibrinolysis

Fibrinolysis is a normal haemostatic response to vascular injury. Plasminogen is converted to plasmin by proteolytic cleavage. The principle plasminogen activator is tissue plasminogen activator (t-PA). Plasmin with the help of other fibrinolytic agents causes degradation of the fibrin clot to yield fibrin degradation products.

Investigation of Disorders of Haemostasis (Screening Tests for Haemostatic Disorders)

Hemorrhagic disorders are caused by: (a) deficiency of clotting factors; (b) platelet deficiency or dysfunction; (c) defective capillaries or (d) excessive fibrinolysis. [Both]

Bleeding Time (BT)

Bleeding time is a test performed to assess platelet function. It is done by puncturing the skin at a specific site on the patient's arm and measuring the time needed for bleeding to stop. A prolonged bleeding time is an indication of platelet dysfunction, thrombocytopenia and other vascular disorders.

Materials

- Sphygmomanometer
- Cleaning swabs
- Disposable lancet
- Filter paper
- Stop watch

Method

The Ivy method is the traditional format for this test:

1. Place a sphygmomanometer cuff around the patient's arm above the elbow.
2. Inflate to 40 mm Hg and keep at this pressure throughout the test.
3. Clean the volar surface of the forearm with 70% ethanol.
4. Make two separate punctures 5-10cm apart with a sterile disposable lancet.
5. Remove the blood at 15 second intervals with filter papers. Avoid touching the wound.
6. Record the time taken for each puncture site to stop bleeding.
7. Calculate the average of the two readings.

Normal Range

The normal range is 2-7 minutes

Interpretation

A prolonged bleeding time is an indication of: (a) platelet dysfunction; (b) thrombocytopenia; (c) other vascular disorders.

Prothombin Time (PT)

The PT test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin). It indicates the overall efficiency of the extrinsic clotting system (factors I, II, V, VII, X).

Reagents

- Patient and control plasma samples. Platelet poor plasma is obtained by centrifugation at 2000 g for 15 min at 4°C (approximately 4000 rev/min in a standard bench cooling centrifuge). The sample should be kept at room temperature.
- Thromboplastin
- $CaCl_2$: 0.025

Method

1. Deliver 200 µl of working solution into a tube
2. Incubate for 5 min at 37°C
3. Add 100 µl of citrated plasma
4. Mix the contents of the tube.
5. Start the stop watch
6. Record the end point
7. Carry on the test on duplicate on the patient's plasma and the control plasma

Results

The results are expressed as the mean of the duplicate readings in seconds.

Normal Range

- With most rabbit thromboplastin the normal range is 11-16 sec
- For recombinant human thromboplastin the range is 10-12 sec

Interpretation

The common causes of prolonged one stage PTs are:

- Administration of oral anticoagulant drugs (vit K antagonists)
- Liver disease
- Vitamin K deficiency
- Disseminated intravascular coagulation
- Rarely, a previously undiagnosed factor VII, X, V or prothombin deficiency or defect.

Activated Partial Thromboplastin Time (APTT)

The test measures the clotting time of plasma after the activation of contact factors but without the presence of tissue thromboplastin. It indicates the overall efficiency of the intrinsic pathway (VIII, IX, XI, XII).

Reagents

- Platelet poor plasma (PPP)
- Kaolin
- Phosphilipid
- $CaCl_2$

Method

- Incubate the APTT kit reagents (R_1,R_2) at 37°C
- Pipette 100 µl of citrated plasma to a tube
- Add 100 µl of R_1
- Mix and incubate for 5 min at 37°C
- Add 100 µl of R_2
- On addition of R_2 start the stop watch and determine the coagulation time

Results

Express the results as the mean of the paired clotting times.

Normal Range

The normal range is typically within 26-40 sec.

Interpretation

The common causes of a prolonged APTT are:

- Disseminated intravascular coagulation (DIC)
- Liver disease
- Massive transfusion with plasma-depleted red blood cells
- Administration of or contamination with heparin or other anticoagulants
- A circulating anticoagulant (inhibitor)
- Deficiency of a coagulation factor other than factor VII

Platelet Count

Platelets can be counted in whole blood using the same techniques that are used for counting red blood cells. For the manual platelet count, formal citrate is used as a diluent. This diluent lyses white blood cells leaving red cells and platelets intact. EDTA anticoagulated blood is used. Platelets are counted in the hematocyometer.

Method

1. Pipette 20 µl of EDTA blood into a tube
2. Add 4ml of formal citrate
3. Mix and fill the counting chamber (hematocytometer) with the diluted blood
4. Put the hematocytometer in a wet petridish for 10 min to allow the platelets to settle down
5. Count under the microscope using the x40 objective
6. The platelets appear as small highly refractile particles if viewed with the condenser racked down
7. Five squares in the hematocytometer are counted

Calculation

Number of platelets in 1 µl of diluted blood:

$$\frac{N}{0.02} = \frac{N}{2 \div 100} = \frac{N \times 100}{2} = N \times 50$$

Number of platelets in 1 µl of whole blood:

$N \times 50 \times 200 = N \times 10000$

Normal Range

$280 \pm 130 \times 10^9/l$

Automated Platelet Count

In automated counters, platelets can be counted in whole blood using the same techniques of electrical or electro-optical detection as are used for counting red cells. A new method for platelet counting by flow cytometry has been developed. Platelets in a blood sample are labeled fluorescently with a specific antibody or combination of antibodies. By measuring the RBC:platelet ratio, the platelet count can be calculated.

Fibrin degradation products (FDPs), D-dimer

These tests are performed to check the fibrinolytic activity. Fibrinogen and fibrin are broken down by plasmin to produce fibrin degradation products (D, E, X and Y). D-dimer is measured by latex agglutination. This is done latex coated with anti D dimer antibodies or antibodies specific for the degradation product we want to detect.

Reagents

D-dimer Kit

- The latex is coated with antibodies specific to D-dimer
- The method is qualitative,semi quantitative
- Sample is EDTA or trisodium citrate blood

FDPs Kit

- The latex is coated with antibodies specific to D, E
- Required serum dilution
- Trisodium citrate blood is used. Sometimes a tube containing an antifibrolytic agent and thrombin is used

Method

1. 1 drop of +ve control with 1 drop of latex
2. 1 drop of –ve control with 1 drop of latex
3. 50 µl of sample with 1 drop of latex
4. Mix for 2 min
5. A –ve result is indicated by no agglutination; FDPs are less than 10µg/ml
6. A +ve result is indicated by the presence of agglutination; FDPs are more than 10µg/ml
7. In case of a positive result, the semi quantitative test should be performed

Normal Value

The normal value of FDPs is less than 10µg/ml

Semi Quantitative Test

1. Obtain four tubes
2. In the first tube: add 400 µl of buffer + 100 µl of serum
3. Add 200 µl of buffer to tubes 2,3 and 4
4. Tubes 1,2,3 and 4 have the following dilutions respectively: $^1/_5$, $^1/_{10}$, $^1/_{20}$, $^1/_{40}$
5. Mix a drop of the contents in tube 2 with a drop of latex
6. Mix a drop of the contents in tube 3 with a drop of latex
7. Do the same for tube 4
8. If agglutination occurs, this means that FDP concentration is more than the reciprocal of that tube's dilution
9. E.g. if agglutination was present in the 3rd tube; FDPs are more than 20 µg/ml

Note:
Fibrinogen is high in the following conditions:

- DIC
- Deep vein thrombosis
- Pulmonary embolism
- Pregnancy

Automated Coagulation Analyzers

In modern laboratories, coagulation tests are carried out by means of automated coagulation analyzers. These instruments independently perform coagulation tests including; PT, APTT, fibrinogen, factor assays, plasminogen and other tests. Most machines depend on principles like Inductive dual magnetic circuits. Other instruments use different techniques.

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