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UNIT-II

Counting of Blood Cells: Neubaeur counting chamber

Total RBC count: diluting fluids, Macro dilution / Micro dilution technique, Falsely Low and High Counts, Normal values

Total WBC count: diluting fluids, Macro dilution / Micro dilution technique, Falsely Low and High Counts, Normal values - correction for T-WBC

Absolute Eosinophil count –

Differential Leucocyte count: Granulocyte / Agranulocytes, Morphology / Function, Staining Technique

Platelet Count: Morphological characters / Functions, Direct /Indirect method

Reticulocyte count: Dry/ Wet smear technique.

Haemoglobin: Composition/Normal Values: Determinations: Tallquist/Acid Haematin /Alkaline Haematin, Haldane's Carboxy/Drabkins /Dare, Spencers/ Specific gravity/ Gasometric, Chemical methods.

COUNTING OF BLOOD CELLS

- *NEUBAUER CHAMBER*

INTRODUCTION

- Despite the fact of the recent technical development of scientific laboratories, the Neubauer chamber remains the most common method used for cell counting around the world.
- This article has been written in order to help newbies and experimented researchers to perform a proper cell counting using a Neubauer chamber or Hemocytometer.
- The principles described in this article apply to any cell counting chamber, although the dimensions and volumes of each chamber may differ.
- First, the parts and basic principle of the Neubauer chamber are described
- Second, the article describes how to perform a cell count step by step, in order to achieve reliable and reproducible results. The article describes best practices and recommendations when performing a cell count.

THE NEUBAUER CHAMBER, OR HEMOCYTOMETER

- The Neubauer chamber is a thick crystal slide with the size of a glass slide. (30 x 70 mm and 4 mm thickness)
- In a simple counting chamber, the central area is where cell counts are performed. The chamber has three parts.

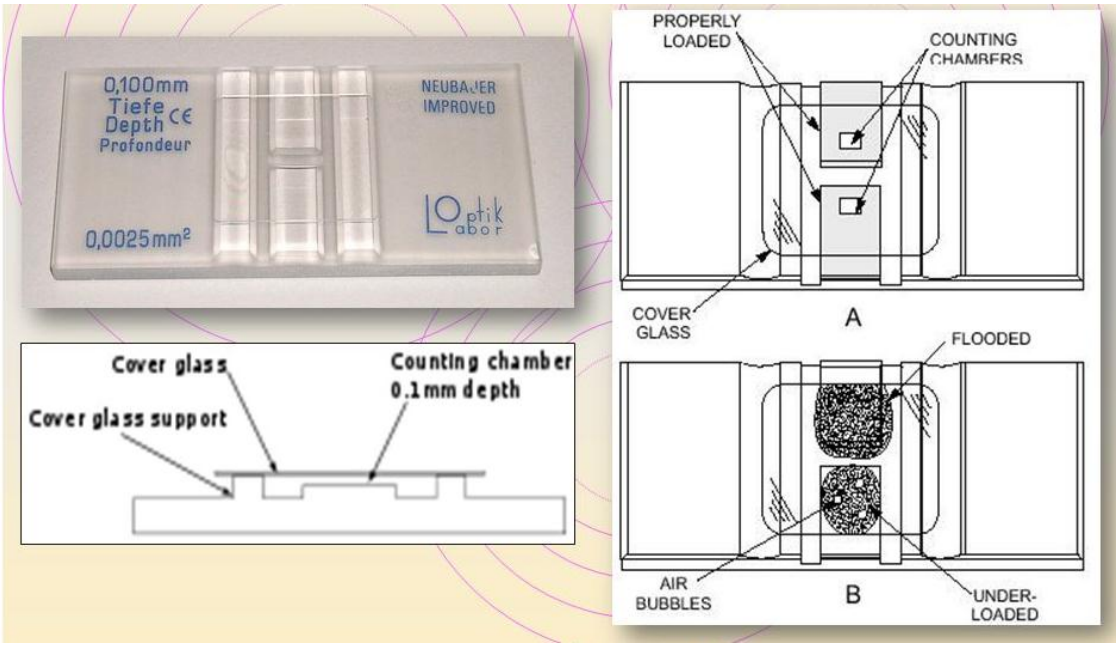

Fig. 1 (a) & (b)

- Therefore, the central square is made of 400 small squares.

CELL COUNT, *STEP BY STEP*

STEP 1. Sample preparation.

i. Depending on the type of sample, a preparation of a dilution with a suitable concentration should be prepared for cell counting.

ii. Above 2.5 million, it is preferable diluting the sample to obtain a final concentration closer to the optimum 1 million per ml. It is important to write down the dilution performed to the original sample.

STEP 2. Introducing the sample into the Neubauer chamber

- Take 10 µl of dilution prepared in STEP 1 with the micropipette.

i. Put the glass cover on the Neubauer chamber central area. Use a flat surface to place the chamber, like a table or a workbench.

ii. Put a disposable tip at the end of the micropipette.

iii. Adjust the micropipette to suck 10 µl. You can adjust it by turning the upper plunger roulette to select the required pipetting volume.

STEP 3. Microscope set up and focus.

i. Place the Neubauer chamber on the microscope stage. If the microscope has a fixing clamp, fix the Neubauer chamber.

ii. Turn on the microscope light.

iii. In case of high cell concentration, it will become very easy to get lost when counting cells. In this case, a counting technique in zig-zag is used.

STEP 4: Concentration calculation

- Apply the formula for the calculation of the concentration

$$\text{Concentration (cell / ml)} = \frac{\text{Number of cells}}{\text{Volume (in ml)}}$$

- The number of cells will be the sum of all the counted cells in all squares counted.
- The volume will be the total volume of all the squares counted.

Since the volume of 1 big square is:

0.1 cm x 0.1 cm = 0.01 cm^2 of area counted.

Since the depth of the chamber is 0,1mm

0.1 mm = 0.01 cm

0.01 cm^2 x 0.01 cm = 0.0001 cm^2 = 0.0001ml = 0.1 µl

RBC COUNT

Diluting Fluid

This should be isotonic so that RBCs are not haemolysed. Normal saline can be used but it may cause crenation of the RBCs and allow rouleaux formation.

One can use

1. Sodium citrate - 3 gm
   Formalin - 1 ml
   Distilled water to - 100 ml
   (cheap and good) Or

2. Hayem's fluid
   Mercuric chloride - 0.5 gm
   Sodium chloride - 1.0 gm
   Sodium sulphate - 5.0 gm
   Distilled water to - 200 ml.

Method

Draw blood to the 0.5 mark in the RBC pipette (Fig). Wipe tip clean and draw diluting fluid to the 101 mark. Shake for 3 minutes. Charge the chamber. Count the RBCs using 40x objective in the 80 smallest squares as indicated in the diagram of the chamber

$$\text{RBC count} = \frac{\text{No of cells counted} \times \text{Dilution factor} \times \text{Depth factor}}{\text{Area counted}}$$

Interpretation

RBC counts are low in anaemia and high in polycythaemia, the causes of these have already been discussed

WBC COUNT

A white cell count (TLC) estimates the total number of white cells in a cubic millimetre of blood. It is important in the diagnosis of disease,especially when accompanied by a differential white cell count.

The diluting fluid: WBC diluting fluid contains a weak acid to lyse the RBCs and a stain for staining the nucleus of WBCs, e.g. Turke's fluid

Glacial acetic acid	1.5 ml
1% aqueous solution of Gentian violet	1.0 ml
Distilled water	98.0 ml

(A pinch of thymol may be added to the diluting fluid to prevent growth of moulds).

Methods

1. Using a WBC pipette (Fig) of a haemocytometer, draw well mixed venous blood or capillary blood and fill till the 0.5 mark. Clean the tip of the tube. Now draw WBC diluting fluid till the 11 mark (or to 0.38 ml of diluting fluid add 0.02 ml of blood with a Hb pipette.
2. Mix the fluid and blood mixture gently avoiding bubbling.
3. Place the coverslip on the counting chamber at the right place.

Shake the fluid-blood mixture and transfer the mixture using a fine bore Pasteur pipette on to the counting chamber (called charging the chamber), taking care that the mixture does not overflow. If it does overflow, wash and dry the chamber to be recharged again.

Calculate the number of cells per cubic millimetre of blood as follows:

$$= \frac{\text{Cells counted} \times \text{blood dilution x chamber depth}}{\text{Area of chamber counted}}$$

(Dilution factor is 20 for there is no mixing of cells till first 1 mark of the WBC pipette, hence 0.5 parts of blood are present in 10 parts of the diluting fluid-dilution factor, then, is 10/0.5 = 20).

Falsely high counts occur due to:

1. Blood taken from an area where there was haemoconcentration.
2. Not wiping away the blood on the outside of tip of the pipette.
3. Blood drawn above the mark in the pipette
   (happens usually when the rubber mouth- piece is too short).
4. Diluting fluid not taken till the requisite mark

Falsely low counts occur due to:

1. Dilution of the blood with tissue fluid due to oedema or squeezing
2. Delay in counting (this does not affect RBCs as much as WBCs, which are reduced by about 15% in 24 hours).
3. Blood not drawn up to the requisite mark.
4. Diluting fluid taken in excess of the requisite mark.
5. Saliva in the mouthpiece running into the upper end of the pipette causing further dilution.

Calculation

$$= \frac{\text{Number of nucleated RBCs} \times \text{TLC}}{100 + \text{number of nucleated RBCs}}$$

ABSOLUTE EOSINOPHIL COUNT (AEC)

(Ref: Textbook of Practical Physiology (4th Edition, University Press) G.K.Pal & Pravati Pal)

INTRODUCTION

Differential count of leucocytes yields the relative number of eosinophils in the leucocyte population. It is possible to find the absolute number of eosinophils in circulation by performing direct and indirect absolute counts. The direct method of absolute eosinophil count (AEC) is done by using the principle of hemocytometry. Indirect AEC is done by calculating the number of eosinophils as a percentage of the total leucocytes present in the circulation. Therefore, for indirect count, two tests should be performed: the differential count and the total count of leucocytes. The sources of error are greater in the indirect count as it multiplies the error of both the methods. The direct count on the other hand is more accurate. The staining properties of eosinophils make the direct count possible.

Structure and Development of Eosinophils

Eosinophils usually have bilobed nuclei. In a stained preparation, the granules take on a deep red or brick-red colour. Eosinophils are distinguished from neutrophils primarily on the basis of granules rather than the number of lobes in the nuclei. In absolute count, other leucocytes and red cells are destroyed, making it easy to distinguish eosinophils.

Development of eosinophils occurs along the same lines as that of other granulocytes (as described in Chapter 9). Production of eosinophils is regulated by GM-CSF and interleukins IL_3 and IL_5.

Life History

Once released into the bloodstream, most eosinophils migrate within 30–60 minutes into extravascular tissues where they survive for 8–12 days. Like neutrophils, eosinophils are mobile cells whose movement is directed by chemotactic factors derived from a variety of sources including mast cells and lymphocytes.

Functions

1. Eosinophils are present in large numbers in parasitic infestations in which they appear to serve an important defence function. The granules of eosinophils contain a number of chemicals. Some of these chemicals directly kill the larvae of the parasites (larvicidal) and also the adult parasites (parasiticidal).

   Granular contents The granules of the eosinophils contain the following chemicals.

   Major basic protein (MBP) The MBP makes 50 per cent of the mass of the granules. It is a potent tissue toxin that kills larvae and adult parasites.

   Eosinophilic cationic proteins (ECP) The ECP is a bactericidal and larvicidal agent.

   Eosinophil peroxidase This enzyme participates in inflammatory activities.

   Aryl sulphatase B This enzyme inactivates leukotrienes that are involved in hypersensitivity reactions. It also inactivates the slow-releasing substance A.

   Lysophospholipase This is a membrane-bound enzyme that causes hydrolysis of intracellular lipoproteins.

   Histaminase It causes degradation of histamine.
2. Eosinophil count also increases in patients suffering from allergic diseases in which exposure to abnormal exogenous or endogenous antigens leads to an immunologic reaction. In these allergic conditions, eosinophils dampen the host's response by limiting the antigen-induced release of mediators of inflammation.
3. Eosinophils are also phagocytic and destroy organisms through oxidative mechanisms similar but not identical to those of neutrophils. Eosinophils can phagocytose bacteria, fungi and inert particles, but are less efficient than neutrophils.

Normal Count

The normal range is 40 to 440 per µl of blood. There is no sex and age variation.

METHODS OF COUNTING

Absolute count of eosinophils is done by two methods: (1) directly by using the principle of hemocytometry and (2) indirectly by studying the smear and total leucocyte count.

Direct Method

Principle

Blood is diluted 10 times in a WBC pipette with a special diluting fluid, which removes red cells and stains the eosinophils. The diluted blood specimen is then charged in a counting chamber and the cells are counted under a high-power objective. The population of eosinophils is then calculated for the undiluted blood.

Requirements

I. Equipment

1. Microscope
2. Hemocytometer (WBC pipette and counting chamber). Three counting chambers are commonly used.
   i) Fuchs–Rosenthal counting chamber
   ii) Neubauer counting chamber
   iii) Speir counting chamber

   Fuchs–Rosenthal counting chamber is preferred because it is specially designed for the eosinophil count. It has a depth of 0.2 mm to accommodate more diluting fluid.

   The Speir chamber is very similar to the Fuchs–Rosenthal counting chamber.

   However, as these chambers are not usually available, the Neubauer chamber is commonly used in our laboratories.
3. Materials for sterile finger puncture
4. Watch glass
5. Filter paper
6. Petri dish

II. Reagent (diluting fluid)

There are three diluting fluids available for eosinophil count: Pilot solution, Randolph solution and Dunger solution.

Pilot solution This is the most frequently used diluting fluid.

Composition

i) Phloxine B (1% solution in water)	: 10 ml
ii) Propylene glycol	: 50 ml
iii) Sodium carbonate solution (10% solution in water)	: 1 ml
iv) Heparin	: 100 units
v) Distilled water	: 40 ml

Function of each constituent

Phloxine	: Stains eosinophil granules
Propylene glycol	: Lyses the red cells
Sodium carbonate	: Lyses all white cells except (with water) eosinophils
Heparin	: Prevents coagulation

Heparin is usually not added to the solution as red cells are lysed by propylene glycol. However, to prevent clumping of red cell fragments, heparin should be added to the solution.

Randolph solution This is very similar to the Pilot solution except that methylene blue is added to help in differentiating other leucocytes (blue) from eosinophils (orange-red).

Dunger solution

Composition

i) Aqueous eosin (1%)	: Stains eosinophil.
ii) Acetone	: Fixes white cells
iii) Distilled water	: Lyses red cells

This solution does not remove other white cells, which appear as grey bodies.

III. Specimen

Capillary blood or EDTA-anticoagulated venous blood is used.

Procedure

1. Clean the watch glass, coverslip, WBC pipette and Neubauer's chamber thoroughly and ensure that these are dry.
2. Take adequate Pilot fluid in a watch glass.
3. Prick the finger tip under aseptic conditions.
4. Suck blood exactly up to the 0.5 mark and clean the tip of the pipette.
5. Suck Pilot fluid up to the 11 mark.
6. Shake the pipette for at least 2 minutes to mix the blood thoroughly with the diluting fluid.
7. Keep the pipette for 15 minutes under the cover of a petri dish lined with moist filter paper.
8. After 15 minutes, take out the pipette, mix the solution by gently shaking the pipette and discard 2–3 drops of the solution from the pipette.
9. Charge the Neubauer's chamber (as described in Chapter 6).
10. Count eosinophils in four WBC squares under the high-power objective of the microscope.
11. Enter the observations in your rough notebook in similarly drawn squares.

Calculation

Dilution is 1 in 20. Therefore, the number of eosinophils counted per mm^3 of blood will be $n \times 50$ (for details see Chapter 9) where n represents the total number of cells counted.

Precautions and Sources of Error

All the precautions observed for pipetting and charging the chamber (described in Chapter 6) should be followed for this experiment too. In addition, the following precautions should also be observed.

1. Counting with capillary blood gives a higher result (about 10–20 per cent more) than with venous blood.
2. Counting should be done within 30 minutes of charging the chamber because eosinophils slowly disintegrate in the diluting fluid.
3. For mixing the contents of the pipette, shake gently to avoid undue rupture of the eosinophil membranes.
4. After diluting the blood, the pipette should be kept under the cover of a petri dish lined with moist filter paper, for 15 minutes (staining time) to prevent evaporation.
5. Use the indirect counting method simultaneously to check the result of direct counting.

Indirect Method

This is one of the ways to check the result of the absolute eosinophil count. It should tally with the value obtained in the differential leucocyte count (relative count). In the indirect method, the value of the total leucocyte count is required in addition to the value of the eosinophil percentage of the differential count.

$$\text{AEC} = \frac{\text{Differential count}}{100} \times \text{Total leucocyte count}$$

For example, if the eosinophil percentage in DLC is 4 and the TLC is 7000/mm^3 of blood, then the AEC = (4/100) × 7000 = 280/mm^3 of blood.

Note: If the results of the direct and indirect methods differ significantly, the absolute count of eosinophils by the direct method should be repeated.

DIFFERENTIAL LEUKOCYTE COUNT (DLC)

TYPES OF LEUKOCYTES:

1. GRANULOCYTE CELL :

a) ***Neutrophil:*** is a cell with acidophilic cytoplasm and fine pinkish red granules, the nucleus is usually lobulated (3-5) lobes, connected by thin chromatin filament .

- Percentage 65-70% from blood
- Number 3000-6000/ mm^3.

b) ***Eosinophil***: acidophilic cell, usually larger than the Neu., it is cytoplasm contain bright red granules or orange the nucleus consist usually 2 lobes or (bilobes).

- Per. 2-4 % from blood
- Num. 150-300/mm^3

c) ***Basophil:*** it is a small cell granules are black or blue in color and over cover most of the cell even the nucleus or (S-shaped).

- Per. 0-1 % from blood
- Num. 0-100 / mm^3

2. AGRANULOCYTE CELL:

a) ***Lymphocyte:*** it is also small cell, the nucleus cover most of the cells &it is round dark violet in color, the cytoplasm is usually blue in color with no granules.

- Per. 32-40 % from blood.
- Num. 1500-4000 / mm^3.
- Lym. is divided into 2 types :T-cell(cytotoxic), B- cell(production of antibodies)

b) ***Monocyte:*** is the largest mature leukocyte the nucleus is usually kidney shape or horse shape.

- Per. 5-8 % from blood.
- Num. 300-600 / mm^3
- Monocytosis: ex: Malaria, Typhus.

The procedure of slide to slide:

i. Place a drop of blood from the finger about 2mm in diameter in the central line of a slide about 1-2 cm from one end.
ii. The spreader is placed at an angle of 40 degrees to the slide and then moved back to make contact with the drop.
iii. The drop should spread out quickly along the line of contact of the spreader with the slide.
iv. The moment this occurred the film should be spread by a rapid, smooth, forward, movement of spreader.

There are 3 stains that are used in differential leukocyte count:

a) Wright stain,
b) Leishman stain.
c) Giemsa stain

Method of staining:

i. The blood film is fixed with methyl alcohol for 2 minutes .
ii. Pour Giemsa stain diluted 1:9 with buffer over the smear for 8-10 minutes.
iii. Wash off with buffer and dry.

The Count:

- The dry and stained film examined without a coverslip under oil immersion objective.
- For differential leukocyte counts choose an area where the morphology of the cells is clearly visible.
- Do differential count by moving the slide in area including the central and peripheral and the smear.
- A total of 200 cells should be counted in which every white cell seen must be recorded in a table under the following heading:
  Neutrophil, Eosinophil, Basophil, Lymphocyte, and Monocyte then find the percentage of each type.

PLATELET COUNT

(Ref: Textbook of Haematoogy, Dr.Tejindar Singh, 3rd Edition, Arya Publications, 2018)

PLATELETS – Morphology, Structure & Functions

PLATELETS

Platelets are fragments of cytoplasm of megakaryocytes.

1. ***Normal count:*** 150–400 × 10^9/l
2. ***Life span:*** 4–10 days. As the platelets age, their size decreases and also there is gradual loss of functional capacity due to depletion of metabolic stores. Elimination half life is 30 hours.

FUNCTIONS

Platelets play an essential role in

- haemostasis
- coagulation
- thrombosis.

Morphology in a smear

In a Romanowsky stained smear, platelets appear as pale pink coloured structures with purple granules with variation in size. (Fig. 1.19) In a normal person there should be 1 platelet for every 10–25 red cells. In a finger prick smear platelets are in clumps, while in EDTA smears, platelets are scattered.

STRUCTURE

- Platelets are disc shaped cells without any nucleus, golgi apparatus or endoplasmic reticulum.

- Size
  - 3.5 ± 0.7 µm diameter
  - thickness 0.9 ± 0.3 µm
  - volume 7 ± 4.5 µm³ (fl).
- It consists of
  - glycocalyx
  - platelet membrane
  - platelet cytoskeleton
  - canalicular system
  - organelle zone.

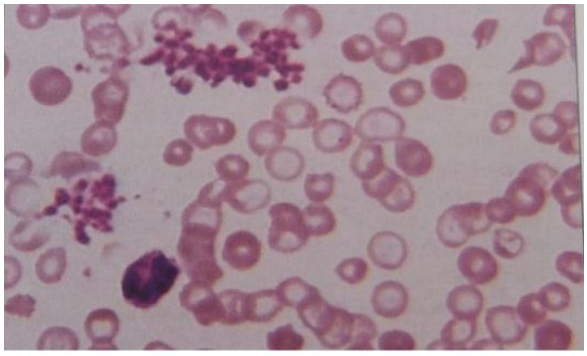

Fig. Platelets: Peripheral smear shows clumps of platelets

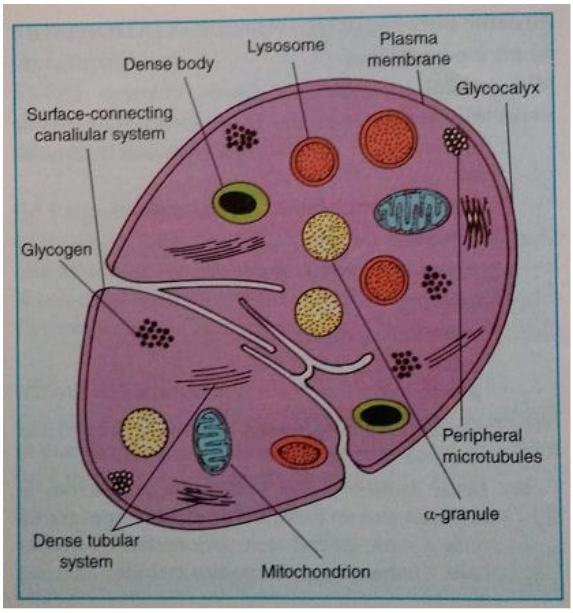

Fig.Ultrastructure of platelet - diagrammatic representation

Preferably, use venous blood for platelet counts. Finger prick may cause clumping of platelets. In small children, however, this clumping can be prevented by thinly smearing Vaseline over the area to be punctured (make sure that there has been no clotting of bood).

The blood is diluted in 1% ammonium oxalate stored refrigerated at 4ºC which haemolyses the RBCs (prepared by dissolving 1 gm of ammonium oxalate in 100 ml of distilled water)

Methods

- Fill blood and diluent (in this case 1% ammonium oxalate) as described for the RBC count and using the RBC pipette. If platelet count is low, a WBC pipette can be used instead.
- Charge the chamber with the help of the pipette employed.
- Using 40x objective with reduced condenser aperture, count the platelets in the same squares as indicated for RBC counting
- Calculate as (if RBC pipette used)

$$= \frac{\text{Cells counted} \times \text{Blood Dilution} \times \text{Chamber depth factor}}{\text{Area of chamber counted}}$$

However, if a WBC pipette is employed, the appropriate formula and method should be used. Platelet counts are made in the small 5 RBC squares only

Platelet count = $N \times 200 \times 5 \times 10$ or $N \times 10000$

Normal platelet counts = 1.5 to 3.5 lakhs / cu mm

Rees-Ecker Method for Platelet Count

Various components of the diluting fluid used have various functions, e.g. citrate prevents coagulation while formaline fixes the platelets and prevents their clumping together. Here, no attempt is made to lyse RBCs. Platelets are identified by their size, shape and dark colour Brilliant cresyl blue (the dye used) provides the background during cell counting. This dye does not stain the platelets and, therefore, is not essential for the counting procedure.

Diluting Fluid

Consists of

Trisodium citrate	- 3.8 gm
Neutral formaldehyde	- 0.2 ml
Brilliant cresyl blue	- 0.1 gm
Deionised water	- 100 ml

Dissolve the ingredients in 100 ml volumetric flask, filter, centrifuge, transfer to a well stoppered bottle and keep at 2-8'C (refrigerate). This fluid if not contaminated will stay good indefinitely. Filter aliquot of the diluting fluid immediately before use.

Procedure

- Take 3.98 ml of diluent (freshly filtered) into a test tube
- Add to the diluent 0.02 ml (20 µ1) of well mixed anticoagulated blood. With the help of a Sahli pipette, wipe out the outer tip of the pipette before dilution. Wash out the contents in the pipette into the diluent tube 3-4 times
- Immediately mix the diluent with the specimen for at least 5 minutes or so.
- Employ the Sahli's pipette for charging either side of the chamber

$$\text{Platelet count/ml or cu mm} = \frac{\text{Number of platelets counted} \times \text{Dilution}}{\text{volume of fluid}}$$

Where

volume of fluid for the 1 sq mm area = 1 x 0.1
= 0.1 ml (cu mm)

Dilution = 200

Rough Estimation of Platelet Count from Stained thin Smear

A well-prepared peripheral blood smear can used to check the results of direct counting. Determine the ratio of platelets to red cells on thin blood smear used for differential leukocyte count. If the average number of platelets is 8 to 25 in 10 fields, it is reported to be adequate, and if it is 0 to 5, it is reported as inadequate.

Causes of Thrombocytopenia

1. *Causes of platelet production failure:*
   Selective megakaryocyte depression
   - Drugs
   - Chemicals
   - Viral infections

   Part of general bone marrow failure
   - Aplastic anaemia
   - Leukaemia
   - Myelosclerosis
   - Marrow infiltration, eg in carcinoma, lymphoma
   - Multiple myeloma
   - Megaloblastic anaemia
2. *Increased destruction of platelets*
   - Acute or chronic ITP (idiopathic thrombocytopenic purpura)
   - Secondary immune thrombocytopenia
     (post-infection, SLE, CLL, and lymphomas)

(Ref: Medical Lab Technology-Methods & Interpretation (Volume 1, 6th Edition, JayPee Brothers) Ramnik Sood)

RETICULOCYTE COUNT

(Ref: Textbook of Haematoogy, Dr.Tejindar Singh, 3rd Edition, Arya Publications, 2018)

RETICULOCYTE COUNT

Reticulocytes are newly formed RBCs released from bone marrow. These take 2 days to mature in bone marrow and are in circulation for about one day during which these can be detected in the peripheral blood. These RBCs are slightly larger with 20% more volume than red cells and contain RNA which stains with basic dyes like Brilliant Cresyl Blue and New Methylene Blue, demonstrating blue filamentous or granular material (Fig. 2.2). The younger the RBC, more is the filamentous material while older reticulocytes stain small granular material. Since the RBCs are stained in a living state in vitro, this staining is known as **supravital staining.** However, reticulocytes stain *polychromatic* with Romanowsky stains and *polychromatophilia* is an indication of high reticulocyte count in a well stained peripheral blood film.

Normal range

- Normal 0.5–2% or red cells
- Cord blood 1–7%

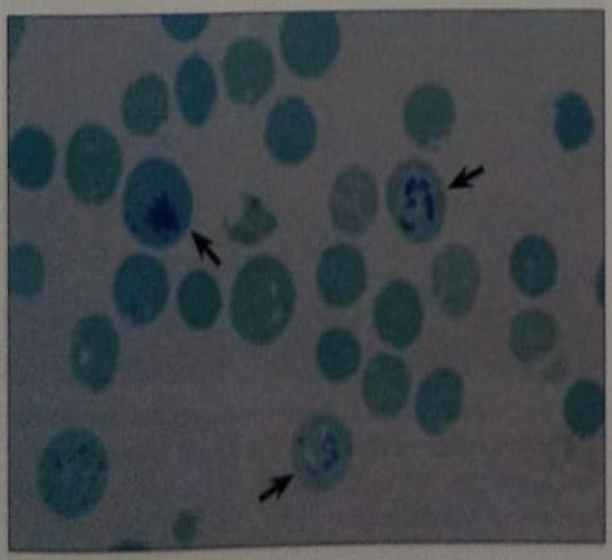

Fig. 2.2 • Supravital stain Brilliant Cresyl Blue demonstrates fine reticulum (residual RNA) in the reticulocytes (↑).

HIGH RETICULOCYTE COUNT

Reticulocyte count indicates the erythropoietic activity of the bone marrow and red cell production. Stimulation of erythropoiesis results in increased reticulocyte release *e.g.* in:

- haemolytic anaemias
- haemolytic crisis
- following therapy in iron/folic acid/B_{12} deficiency anaemias. In such situations, highest counts are observed on 6th/7th day of therapy and reflect marrow response to haematinics.

LOW RETICULOCYTE COUNT

Reduced reticulocyte count (< 0.5%) is observed in diminished erythropoietic activity states like:

- aplastic anaemia
- pure red cell aplasia
- Fanconi's anaemia
- aplastic crisis due to Parvo virus B19 infection in Hereditary spherocytosis and Sickle cell disease.

Reticulocyte Correction for Anaemia

Suppose reticulocyte count in an adult patient with 7.5 gm Hb is 2%, it does not reflect a true marrow response.

Corrected Reticulocyte count

$$= \frac{\text{Patient's Hb} \times \text{Estimated R. count}}{\text{Normal Hb value for that age}}$$

$$= \frac{7.5 \times 2}{15} = 1\%$$

Reticulocyte count and Automated Cell Counters

Reticulocyte count can be carried out using an argon laser based flow cytometer. Reticulocytes are stained directly with Auramine O—a fluorescent dye and are passed through a laser beam. The maturation stages of reticulocytes are defined by the RNA content of the reticulocytes.

HAEMOGLOBIN

(Ref: Medical Lab Technology-Methods & Interpretation (Volume 1, 6th Edition, JayPee Brothers) Ramnik Sood)

HAEMOGLOBIN (HB)

Haemoglobin is the main constituent of the RBCs and carries out the important function of transportation of oxygen from lungs to various parts of the body. To a lesser extent, it transports back carbon dioxide from the body to the lungs. When fully saturated, each gram of haemoglobin holds approximately 1.34 ml of oxygen. The red cell mass of an adult contains approximately 600 gm of haemoglobin, capable of carrying 800 ml of oxygen.

Haemoglobin Estimation: Sahli's Method: (Sahli's Haemoglobinometer) (Fig. 9.3)

This is based on conversion of haemoglobin to acid haematin, which has a brown colour. Fill haemoglobin tube till 20 mark with N/10 HCl. To this, add blood sucked till the specific mark (20 µl) on the haemoglobin pipette and wait for 5-45 minutes. During this time keep stirring the mixture of acid-blood in the tube. Add distilled water until a match is obtained with the brown glass standard (comparator) provided. Read the lower level of fluid meniscus on gm% side of the tube. Report haemoglobin in gm/100 ml of blood. If haemoglobin is less than 2 gm%, take double the quantity of blood and divide the result by 2. If haemoglobin concentration is extremely high dilute blood with equal amount of normal saline, take the reading and multiply by 2. This method, however, does not estimate carboxy-haemoglobin, methaemoglobin and sulph-haemoglobin. Non-haemoglobin substances (protein, lipids) in plasma and cell stroma may influence the colour of blood diluted with acid. It is, therefore, not a very satisfactory method.

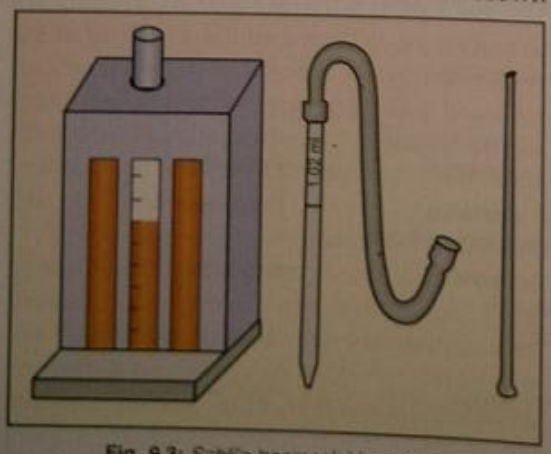

Fig. 9.3: Sahli's haemoglobinometer, middle: Hb pipette, right: Stirrer

Cyanmethaemoglobin Method

(DRABKIN'S SOLUTION AND THE STANDARD AVAILABLE FROM CORAL CLINICAL SYSTEMS, GOA)

Drabkin's Reagent

In 1000 ml of deionized water are mixed:

- Potassium ferricyanide: 400 mg
- Potassium dihydrogen phosphate: 280 mg
- Potassium cyanide: 100 mg
- Nonidet (nonionic detergent): 1 ml

This reagent can be stored in a polythene container. Concentrated stock solutions can also be prepared and diluted accordingly when needed.

Pipette carefully and take care not to discard cyanide solutions into sinks or receptacles containing acid (to prevent formation of hydrocyanic acid). To 5 ml of Drabkin's solution, add 20 µl of blood. Mix well. Read in a photocolorimeter at 540 nm (green filter).

For this procedure, certified standard haemoglobin solution may be obtained from reputable laboratory supply firms. By diluting the known standard haemoglobin solution, a graph (linear) may be obtained by plotting the known Hb