University of Diyala/ College of Medicine
Department of Physiology
Physiology Lab

Platelets (Thrombocytes) Count

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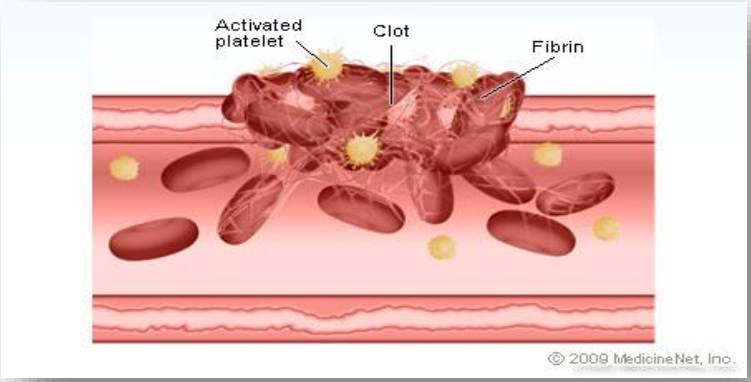


Outlines

- Purposes of Platelets Count Experiment.
- ***Introduction:*** Platelets Physiology and Functions.
- Methods, Procedure, and Calculations.
- Some Applied Aspects.

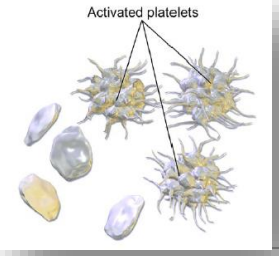
Purposes of Platelets Count Experiment

1. Platelets count is part of a complete blood count (CBC).Total Platelets count tells you whether or not you are suffering from Thrombocytosis a.k.a. Thrombocythemia (i.e. an increase in the no. of platelets or Thrombocytopenia (i.e. a decrease in the no. of platelets)

*** "a.k.a" stands for "*a*lso *k*nown *a*s"**

2. To learn how to do the manual method in the lab to get the number of platelets.

3. To get an idea about the possible causes of abnormal platelet count.

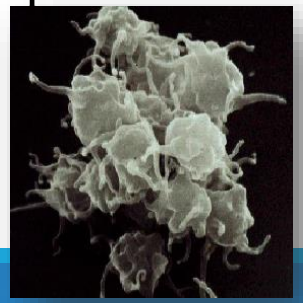


Platelets Physiology

- ***Platelets*** are not cells (but still considered as a part of the blood cellular elements); rather, they are very small, irregularly shaped, non-nucleated fragments from large megakaryocytes.
- The mature platelets are non-nucleated cells with a minute granules present in the cytoplasm and there is no pigment present in platelets.
- ***Shape:*** Shape is spherical or rod and they become oval or disc shaped when inactivated (see figure below).
- ***Size:*** On an average, the size of the platelets is 2 – 4 μm.
- ***Life Span:*** The average lifespan of platelets is 3-10 days. Platelets are eliminated from the circulation mainly by the tissue macrophage system in the spleen.
- ***Normal Range:*** About 150,000– 450,000 cells are present per cubic millimeter (cmm) of blood.

Resting Platelets

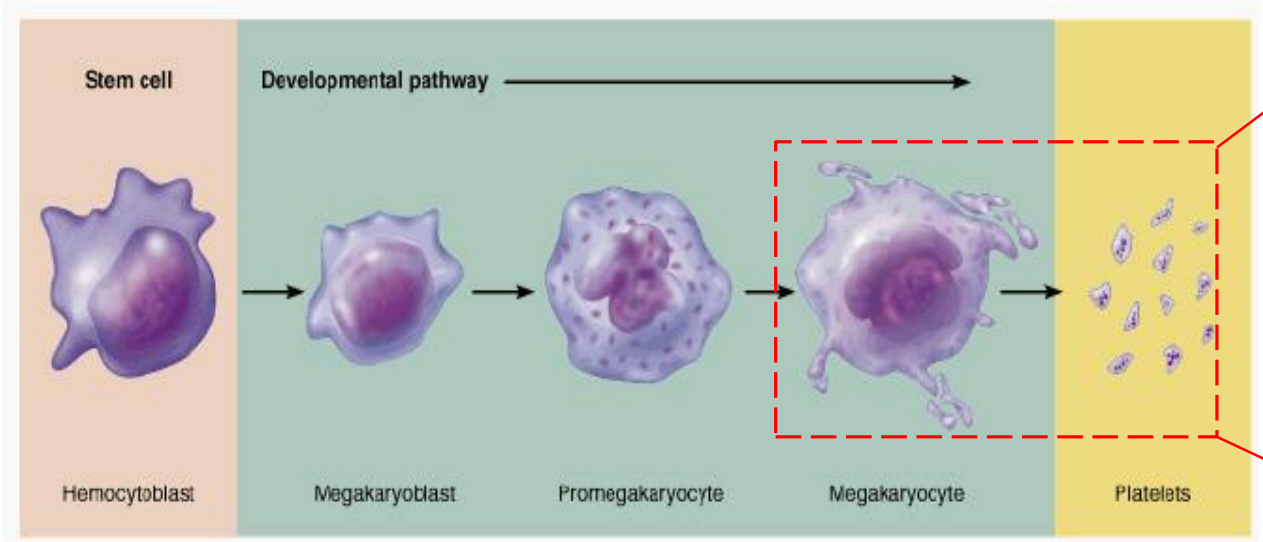
After hitting collagenous fiber

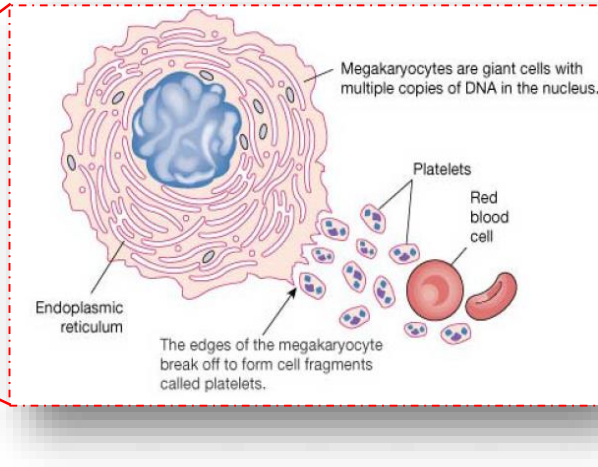


Activated Platelets

Development Pathway of the Platelets

➢Platelets formation (thrombopoiesis) occurs in bone marrow, by budding off from megakaryocytes. Each megakaryocyte produces between 5,000 and 10,000 platelets.

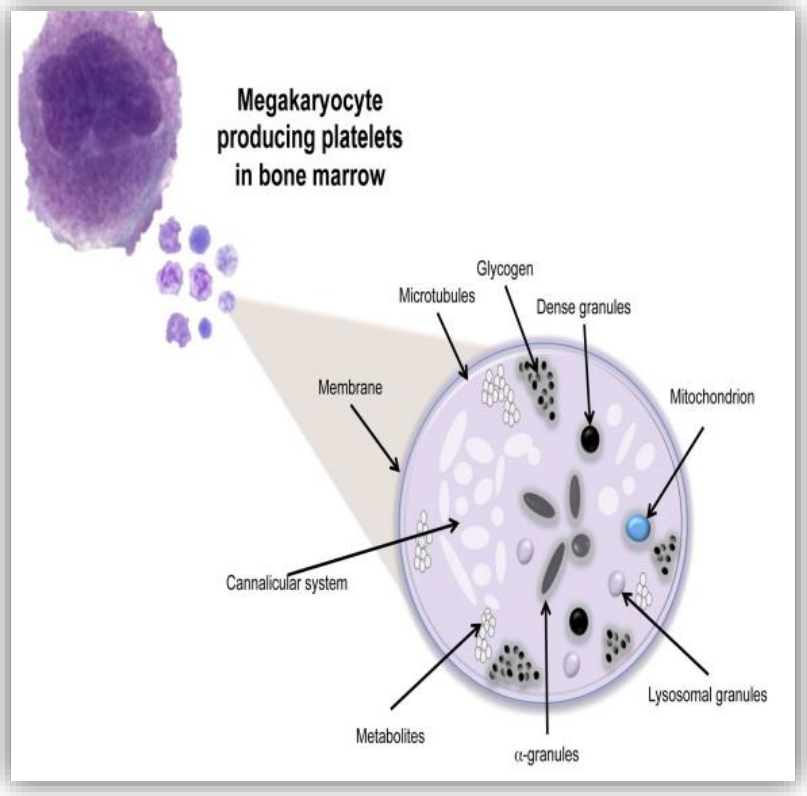




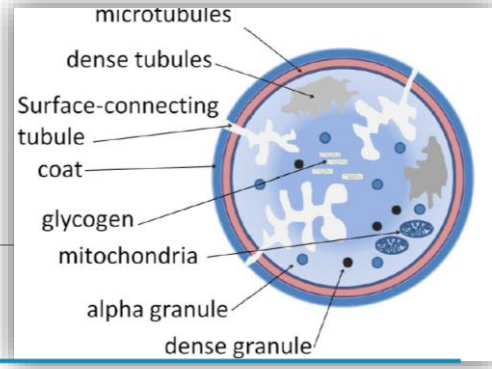
Structure of Platelets

Structure of Platelets

- Cell Membrane
- Microtubule
- Cytoplasm



Structure of Platelets



1. Cell Membrane

It is 6 nm thick and contains:

- Carbohydrates , Proteins(***glycoproteins***), and lipids (***phospholipids***, cholesterol and glycolipids).
- Out of all ***glycoprotein*** and ***phospholipids*** are functionally important.

Plasma Membrane

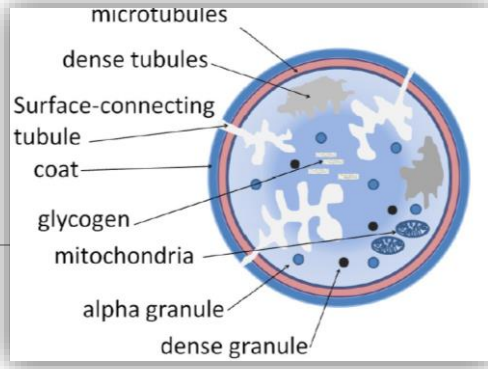
Contains:

- ***Glycoprotein receptors*** for : collagen, fibrinogen, and vessel von-Willebrand factor.
- ***Phospholipids:*** include platelet factor 3.

Glycoproteins

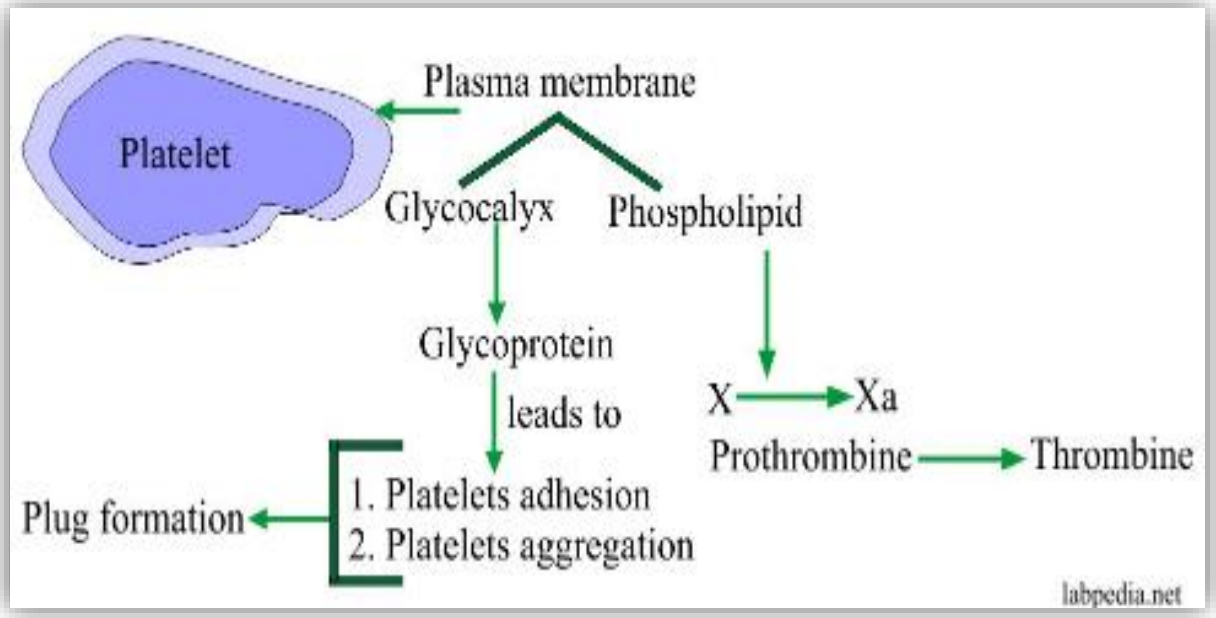
- ***(1)***Prevents the adherence of platelets to normal endothelium, ***(2)***Accelerates the adherence of platelets to collagen and damaged endothelium in ruptured blood vessels, and ***(3)***Forms a receptor for ADP and thrombin.

Structure of Platelets



Phospholipids

- Accelerate clotting reaction.
- Form precursors for THROMBAXANE A2 & PROSTAGLANDINS.



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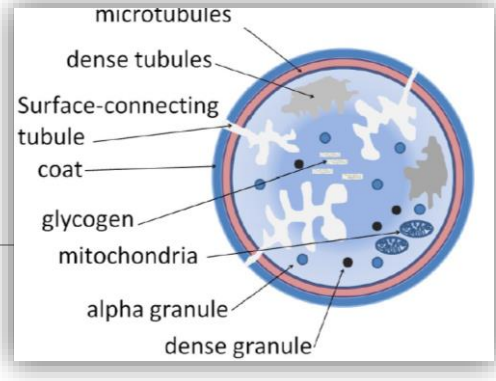
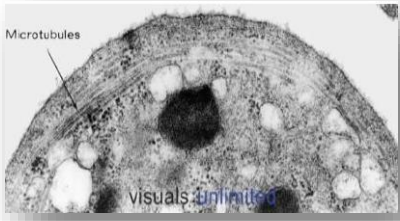


2. Microtubule

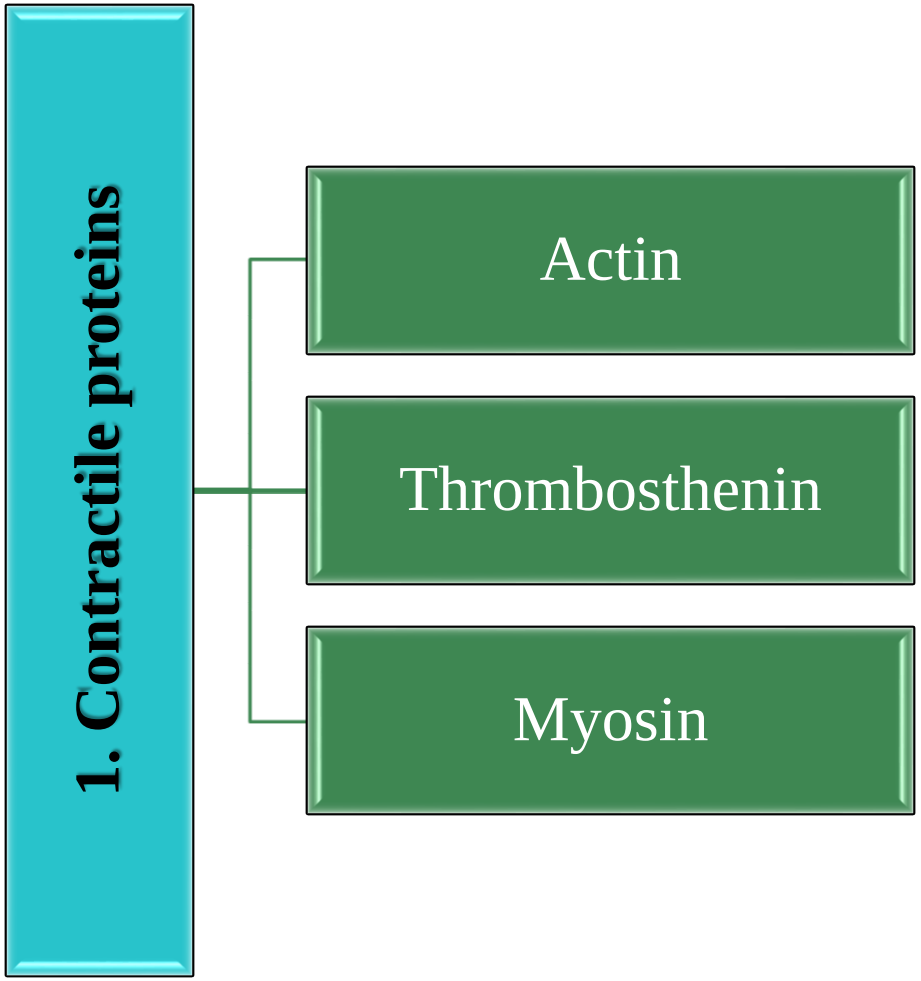
- Made up of tublins (proteins)
  It responsible for structural support for inactivated platelets.



3. Cytoplasm

- Contractile proteins, Golgi apparatus, Endoplasmic reticulum, Mitochondria, Lyzosomes, Glycogen granules, Enzymes, Chemical substances, and Granules.

Structure of Platelets : Cytoplasm



Contractile Proteins enable activated platelets to change their shape.

Structure of Platelets : Cytoplasm

2. Golgi apparatus &Endoplasmic reticulum	• Synthesis of enzymes • Calcium store
3. Mitochondria	• Synthesis of ATP and ADP
4. Lysosomes	• Containing hydrolytic enzymes
5. Glycogen granules	• For production of energy anaerobically
6. Enzyme system	• Synthesize prostaglandins from phospholipids of the Platelet membrane.
7. Chemical substances	• Calcium ions, Mg-ions, ATP, ADP.

Structure of Platelets : Cytoplasm

8. Types of granules:

Alpha Granules

- Contain secreted proteins
- Clotting factors, Fibrin stabilizing factor XIII, and Platelet derived growth factor.

Dense Granules

- Contain non-protein substances.
- ATP, ADP, and Ca++ & serotonin.

Properties of Platelets

Adhesiveness

Injury to blood vessel- Vascular Constriction

1. Platelets become **sticky** and **adhere** to the collagen matrix in sub-endothelium.

2. ***Factors responsible*** are collagen,thrombin, ADP

,thromboxaneA2, Ca ion& Von-WillebrandFactor

Aggregation

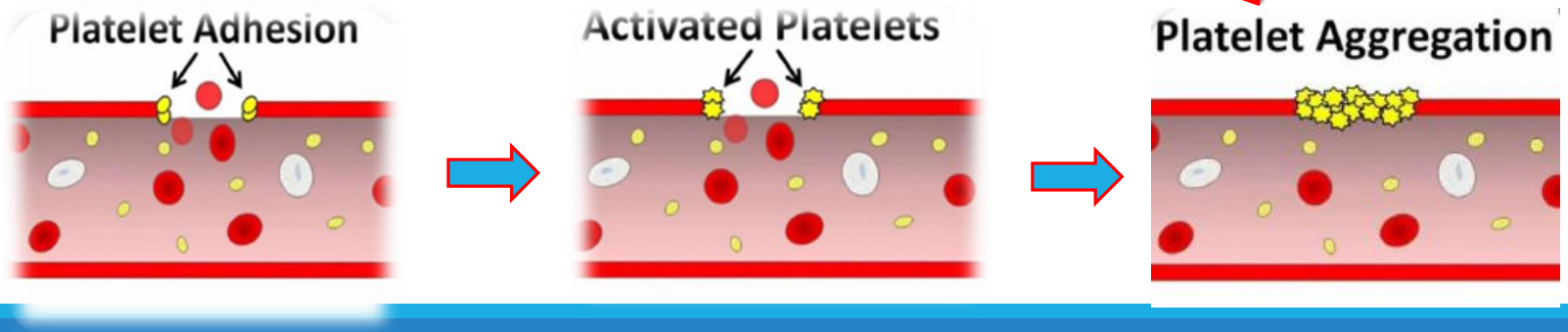
1.Property to stick to each other.

2.Factors responsible are ADP & ThromboxaneA2.

Agglutination

1. Property of clumping together of platelets.

2. Its due to ***Agglutinins of the platelets.***



Functions of Platelets

1. Role in Hemostasis

- Temporary hemostatic plug by platelets due to its property of adhesiveness & aggregation. Definite hemostatic plug –also initiated by platelets.

2. Role in clot formation

- Play role in formation of intrinsic prothrombin activator. It is responsible for onset of blood clotting.

3. Role in clot retraction

- Contraction of contractile proteins.
- Responsible for clot retraction & wound healing.

4. Role in repair of injured blood vessels

- Platelet derived growth factor (PDGF) in cytoplasm of platelet for repairing of endothelium.

5. Role in defense mechanism.

- Due to the property of agglutination, platelets are capable of phagocytosis.
- Mainly in phagocytosis of carbon particles, viruses & immune complexes.

Platelets Count Indications

- To diagnose the cause of petechial hemorrhage in the skin.
- To find the cause of spontaneous bleeding.
- This is advised in a patient on chemotherapy.
- This is advised in case of bone marrow failure.
- Platelets count is of value in thrombocytopenia seen in: uremia, liver diseases, and malignancies.

Platelets Count

Definition

- The calculated number of platelets in a volume of blood usually expressed as platelets per cubic millimeter (cmm) of whole blood.

Principle

- Practically, counting the large amount of Platelets in a sample of blood directly under the microscope is highly impossible. So, they are counted by using hemocytometer or Neubauer chamber. For this, the blood sample is diluted (usually in 1:200 ratio) with the help of isotonic diluting fluid that does not cause any damage to the platelets whereas causes the lysis of red blood cells. Then the diluted blood charged on hemocytometer and the cells are counted in the areas specific for Platelets count.

Methods

- Automated Method (Automated hematology analyzers).
- Blood smear.
- Manual Method (We are going to use this method in the lab).

Manual platelets Count Materials and Instruments

1. Whole fresh blood (using EDTA or heparin as an anticoagulant) or capillary blood can be used.
2. RBC Pipettes.
3. Haemocytometer “Neubauer” chamber is the counting chamber with a cover slip. The same counting chamber is also used for counting RBC and total WBCs.
4. Ammonium oxalate 1% (w/v) in distilled water. Store in refrigerator and filter before use
5. Microscope.
6. Petri dish.
7. Pipette rotator (mixer).
8. Filter paper.
9. Lancet, Alcohol 70%, and Cotton.

Procedure

1. Using two RBC Pipettes. Withdraw blood to exactly 0.5 mark; then dilute to 101 mark with ammonium oxalate(dilution 1:200).
2. Place the pipettes on a pipette rotator for 10-15 minutes to ensure a complete hemolysis of RBCs.
3. Discard the first 3-4 drops from RBC pipette and fill one side of the chamber (as you did in RBC and WBC experiments) . Repeat, using the second RBC pipette and fill the other side of the chamber.
4. Place the chamber in a moist petri dish that has a wet filter paper at the top of it and leave the chamber for 20-30 min (this allows the platelets to settle and prevents evaporation of fluid).

Procedure

5. Put the chamber on the microscope stage and exam under power 10x. The background appears black with the WBCs, platelets, debris and markings of the chamber giving an illuminated appearance.

6. Then change the examination lens to that of power 40x; the platelets appear round or oval bodies with alight purplish sheen. When focusing up and down with the fine adjuster, platelets may be seen with one ore more fine processes.

7. Platelets are counted in the large central square that contains 25 small squares.

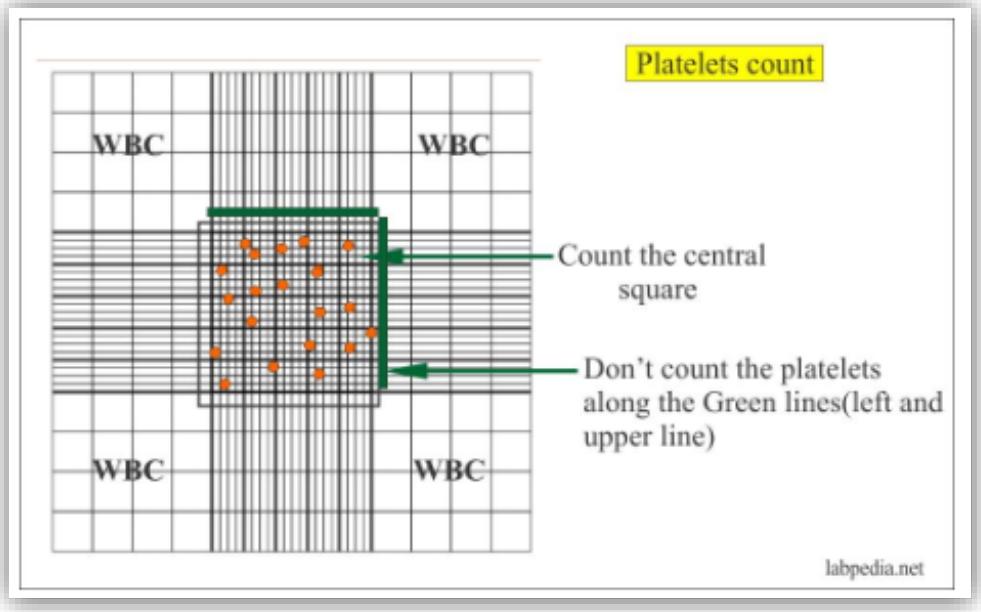
8. Count the no. of platelets in both sides of chamber. The total number of the platelets on each side should agree with the other side by +/- 10 when the platelets count is in the normal range. Add two counts to other and determine the average number of platelets .

Calculations

- **Platelets\ c.mm = N X dilution \volume.**

N : average no. of plat. per c.mm, Dilution : 1:200, Volume: the volume of diluted blood used, which is areas (1mm^2)x depth(0.1mm) = 0.1 c.mm.

Platelets\ c.mm = N x 200 x 10=N x 2000



Tips to Minimize the Errors in Platelets Counting

- Avoid the platelets clumping.
- Avoid the micro clot formation.
- Run the test in duplicate and then get the average of the two results.
- If taking blood from the finger then don’t squeeze the finger.

Precautions to be Taken

- Glassware must be scrupulously cleaned . Debris and dust are the main sources of error as they are easily mistaken for platelets.
- The diluting fluid must be filtered just before use to remove particles.
- If venous blood is used, the platelets must be counted within 3 hours. Delay causes disintegration and clumping of platelets.
- Blood should be rapidly diluted ,this is essential to prevent clumping

Precautions to be Taken

- Blood must be thoroughly mixed with the diluent by shaking the contents at least for 10 minutes. Inadequate mixing results in clumping of platelets.
- The finger should not be squeezed excessively to collect blood .
- The charged chamber should be kept for 15 minutes under petri dish to prevent evaporation and for the cells to settle down.
- If other hematologic tests are to be done with platelet count ,and blood is used from the same puncture ,take blood for the platelet count first.

Sources of Error

- Light adjustment is critical. If the condenser is not lowered, it will fade out the platelets.

- Bacteria and debris can be misinterpreted as platelets. This type of artifact is generally much more refractile than platelets.

- Clumping of platelets sometimes occurs, and if so the specimen must be recollected. EDTA is the anticoagulant of choice for preventing platelet clumping.

- General hemocytometer errors, i.e. overloading chamber, counting wrong borders.

Applied Aspects

Normal Range

- 150,000– 450,000 cells per cubic millimeter (cmm) of blood.

Physiological Variation

- Age , sex, food, exercise , and altitude.

Pathological Variations

- **Thrombocytopenia** when the count is less than 100,000/cmm
  Thrombocytosis when the count is more than 400,000 /cmm.
  Thrombocythemia when the count is above 750000-one million /cmm

Thrombocytopenia: Mechanisms Leading to Thrombocytopenia

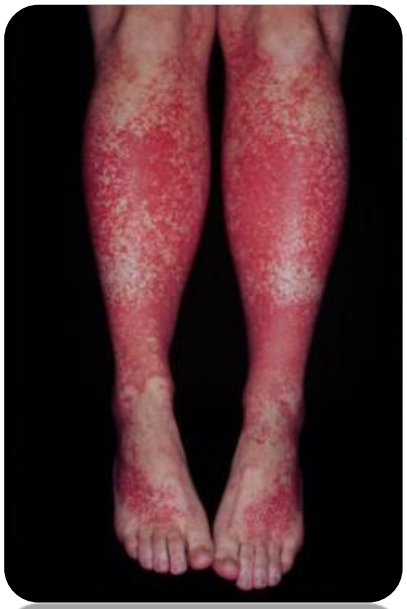
- This may be due to decreased production of the bone marrow (Failure or infiltration of the bone marrow by tumors or fibrosis).
- Destruction or sequestration of the platelets by hypersplenism.
- Antibodies destroying the platelets.
- Destruction of the platelets by infection or drugs.
- Increased utilization of disseminated intravascular coagulopathy.
- In severe hemorrhage which leads to loss of platelets.
- Large blood transfusion leads to a dilution effect.

Causes of Thrombocytopenia or Decreased Platelets

- Idiopathic thrombocytopenia ITP
- Leukemias, carcinoma, and myelofibrosis. This is due to the infiltration of the bone marrow
- Anemias like pernicious, aplastic, and hemolytic
- After a massive blood transfusion
- Bacterial and viral infection
- Chemotherapy treatment
- HIV infection, Renal failure, and toxemia of pregnancy, eclampsia, and Hypersplenism

Signs and Symptoms of Thrombocytopenia

Main Signs and Symptoms of low platelets



- Superficial bleeding into the skin that appears as a rash of pinpoint-sized reddish-purple spots (petechiae), usually on the lower legs.
- Prolonged bleeding from cuts.
- Spontaneous bleeding from gums or nose.
- Blood in urine or stools.

Thrombocytosis when the count is more than 400,000 /cmm.

Thrombocytosis or Increased Platelets: Causes

- Malignant tumors like leukemia, and lymphoma
- Polycythemia vera
- Splenectomy
- Iron deficiency anemia
- Autoimmune diseases like rheumatoid arthritis
- Chronic pancreatitis and inflammatory bowel disease
- Tuberculosis

The term “Thrombocythemia" is preferred when the cause of a high platelet count isn't known. The condition sometimes is called primary or essential thrombocythemia.

Why is Platelet Counting Difficult?

They are small and difficult to discern.

Their adhesive character-attach readily to glassware, particles or debris in the diluting fluid.

They clump easily.

Not evenly distributed in the mixture of blood and diluting fluid.

They readily disintegrate in the blood diluted with fluid making it difficult to distinguish them from debris.

Therefore unless carefully done ,accurate counting of platelets becomes impossible.

Remember that:

1. The patient may develop spontaneous bleeding when the count is **< 20,000 /cmm**.
2. Platelets counts > 50,000 /cmm usually shows no bleeding.

Happy Counting!