Histochemistry

(Cell biology and Histochemistry)

كود

(practical part)

First semester(الفصل الدراسي الاول)

اعداد

د. مريم محمد جاد الرب

كلية العلوم

قسم علم الحيوان

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الرموز المستخدمة

نص للقراءة والدراسة

اسئلة للتفكير والتقييم الذاتي

رابط خارجي

- **رؤية جامعة جنوب الوادي ٢٠١٨ -٢٠٢٣**

التميز في التعليم العالي لبناء تنمية مستدامة في صعيد مصر

- **رسالة جامعة جنوب الوادي ٢٠١٨ -٢٠٢٣**

تسعي جامعة جنوب الوادي الى إعداد الخريجين لممارسة مهنية وبحثية منافسة إقليميا وعالمياً من خلال قدرة مؤسسية وفاعلية تعليمية جاذبة وداعمة تمكن الطلاب من اكتساب مهارات متطورة، وباحثين قادرين على تطوير تخصصاتهم بتقديم بحوث إبداعية وتطبيقية، وتقديم خدمات مجتمعية وبيئية متميزة تسهم في التنمية المستدامة من خلال بناء شراكات استراتيجية فاعلة وتعزيز القيم الوطنية والهوية الثقافية، والتطوير المستمر لبرامج وكليات الجامعة وإداراتها وتأهيلها للاعتماد، ورفع جاهزية وتنافسية الجامعة واستقلال فرعيها والتوظيف الأمثل للموارد.

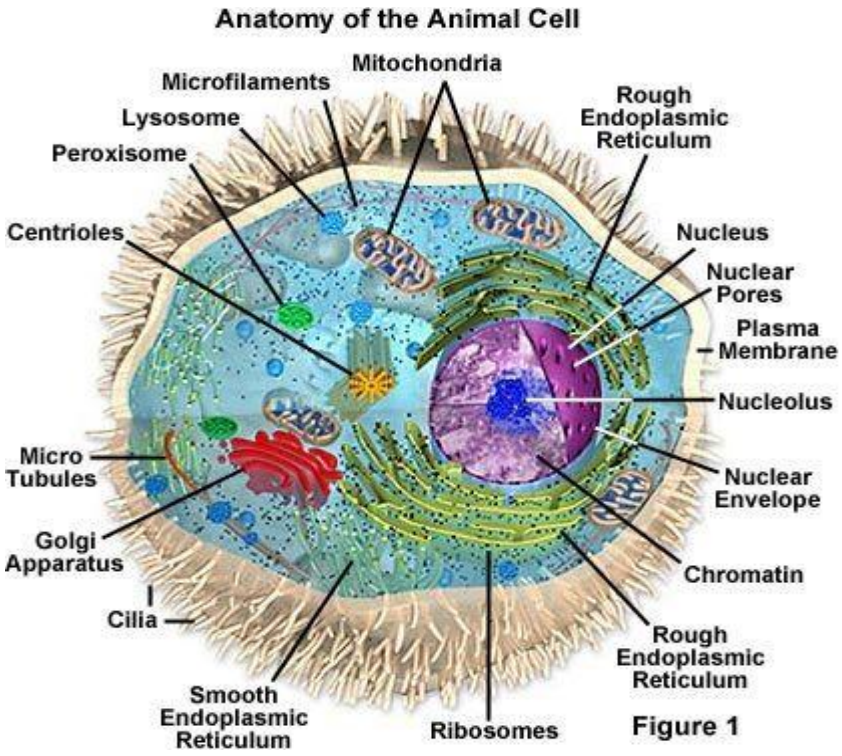
- **رؤية كلية العلوم ٢٠١٨ -٢٠٢٣**

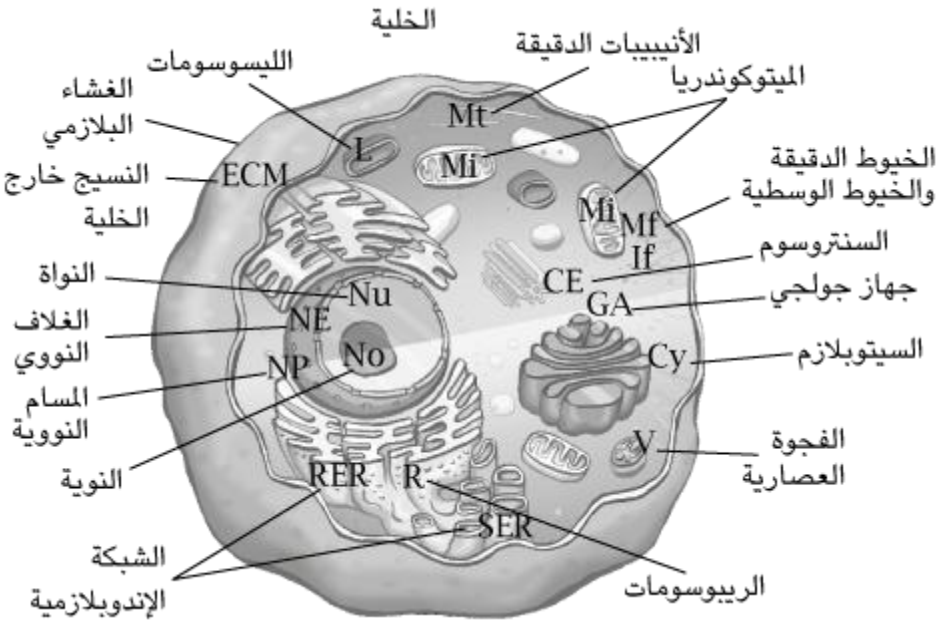
التميز في تعليم العلوم الأساسية والبحث العلمي للمساهمة في التنمية المستدامة

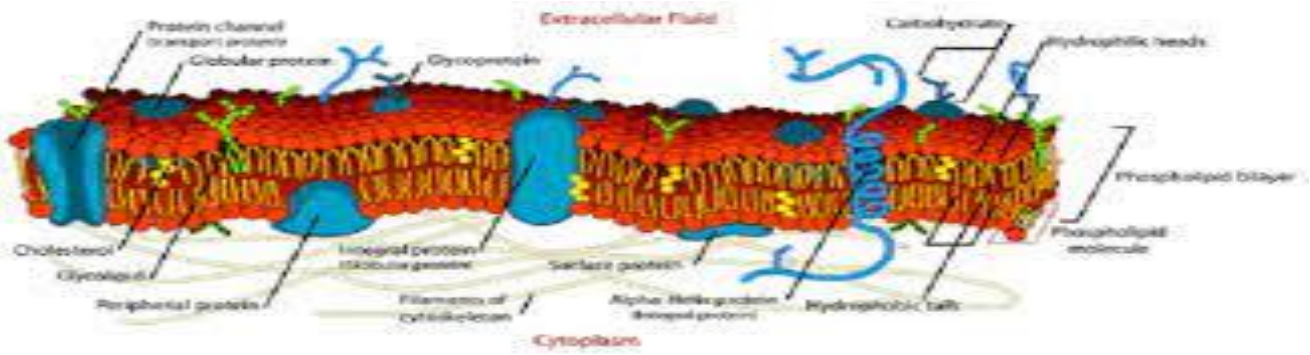
- **رسالة كلية العلوم ٢٠١٨ -٢٠٢٣**

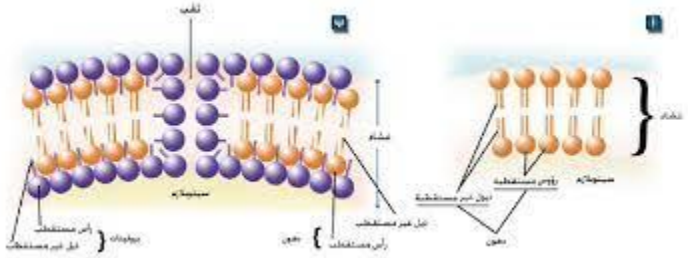
تقديم تعليم مميز في مجالات العلوم الأساسية وإنتاج بحوث علمية تطبيقية للمساهمة في التنمية المستدامة من خلال إعداد خريجين متميزين طبقا للمعايير الأكاديمية القومية، وتطوير مهارات وقدرات الموارد البشرية، وتوفير خدمات مجتمعية وبيئية تلبي طموحات مجتمع جنوب الوادي، وبناء الشراكات المجتمعية الفاعلة.

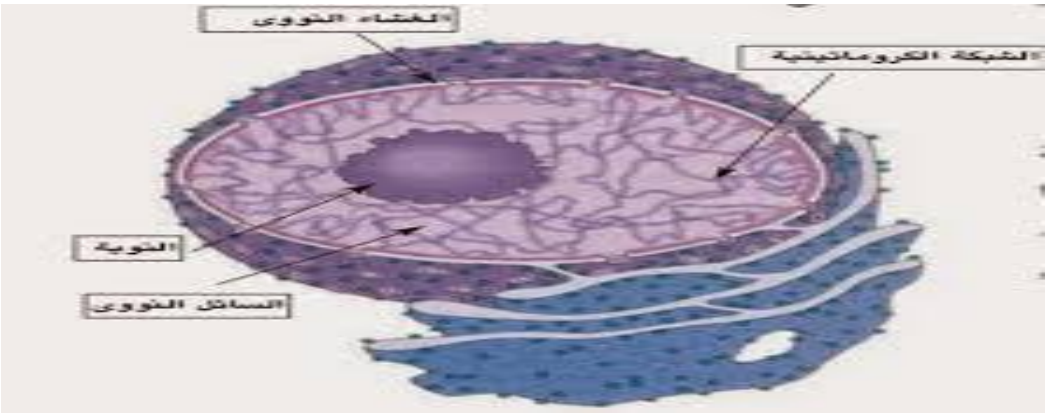
Cell biology

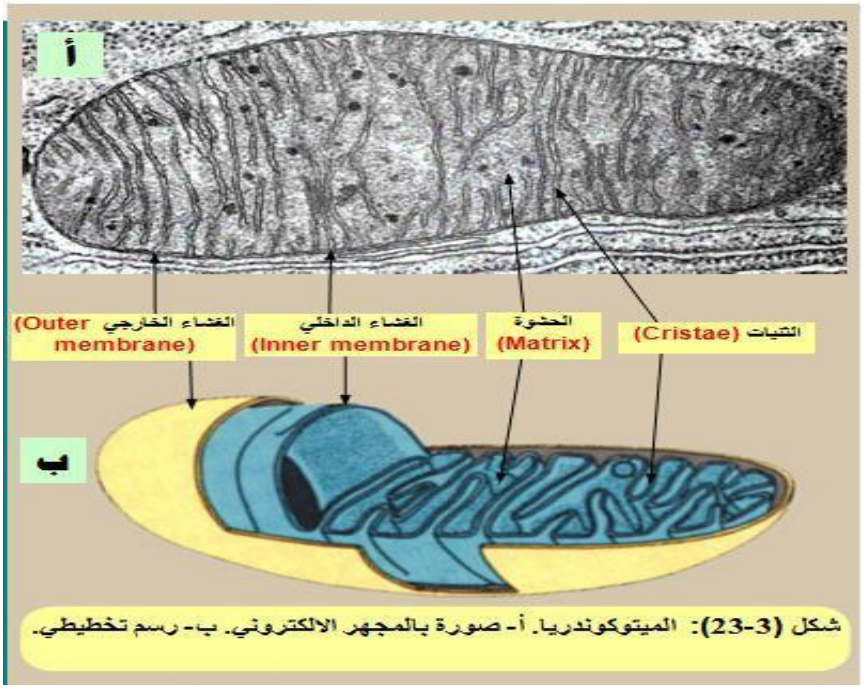




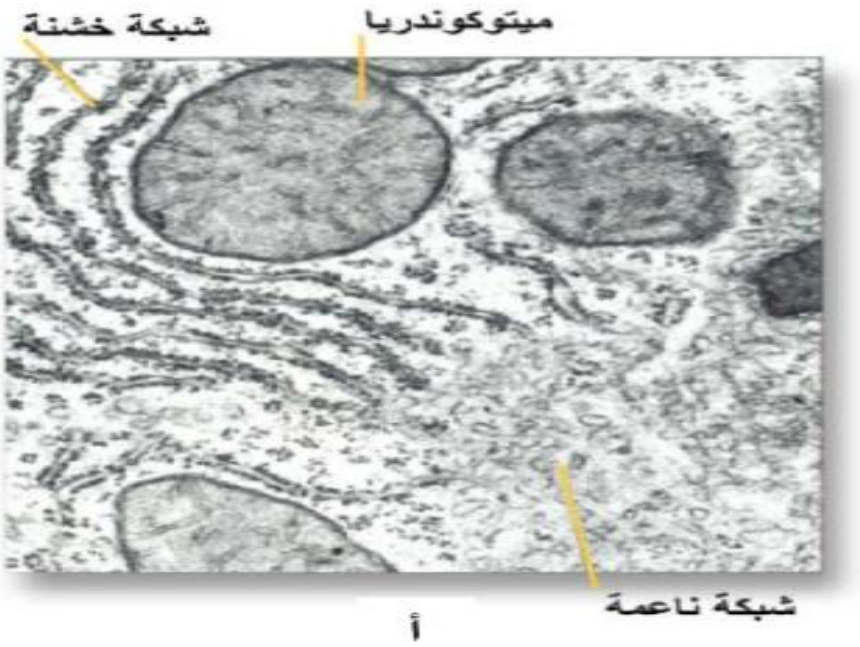

Extracellular Fluid
Protein channel
Globular protein
Glycoprotein
Carbohydrate
Hydrophilic heads
Phospholipid bilayer
Phospholipid molecule
Cholesterol
Glycolipid
Peripheral proteins
Integral proteins
Surface protein
Hydrophobic tails
Cytoplasm







شكل (3-23): الميتوكوندريا. أ- صورة بالمجهر الالكتروني. ب- رسم تخطيطي.


شبكة خشنة
ميتوكوندريا
شبكة ناعمة

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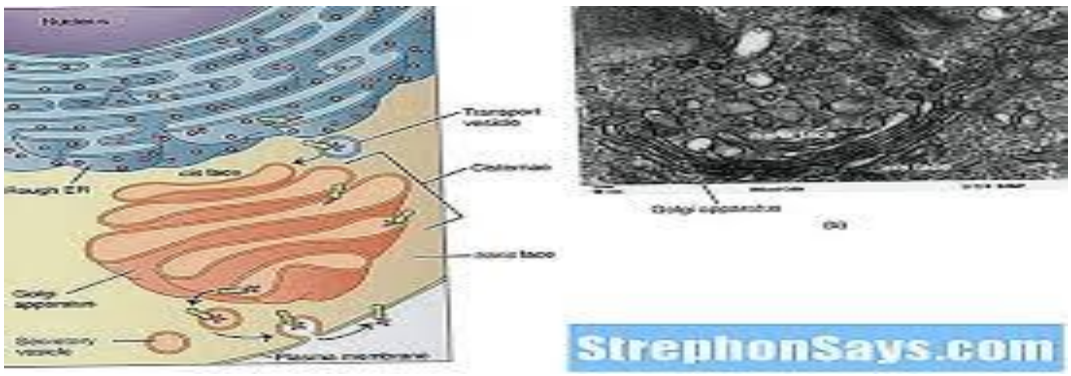

Transport vesicle
Cisternae
Golgi apparatus
Secretory vesicle
Plasma membrane
StrephonSays.com

Histochemistry: is science which studies the patterns of presence and distribution of different

Chemical compounds in tissues and cells of the body, and the role they play in various vital

Activities.

Preparation of tissues for study

There are different methods used, however the basic principles are similar • It usually

Involves hardening of the tissue followed by sectioning (cutting)

- Paraffin technique - Freezing technique- Vital preparation.

1. Paraffin Tissues are hardened by replacing water with paraffin

2. Freezing technique: Water-rich tissues are hardened by freezing and cut frozen

3. Vital preparation is a good method for study the effects of drugs on the living tissues.

The light microscope is not suitable for examining vital specimens. Phase contrast

microscope is used to study the unstained vital preparation.

Protocols followed in Histotechniques

1. Identification & Labeling of the specimen

2. Fixation

3. Dehydration

4. Clearing

5. Impregnation (infiltration)

6. Embedding

7. Section cutting

8. Staining

9. Mounting

Animals should be anesthetized and subjected to cardiac perfusion with saline sol.

Fixation:

This is the process by which the constituents of cells and tissue are fixed in a physical and

chemical state so that they will withstand subsequent treatment with various reagents with

minimum loss of architecture.

This is achieved by exposing the tissue to chemical compounds: fixatives

• Fixatives prevent autolysis and bacterial decomposition and preserves tissue in their natural

state and fix all components

Tissue fixatives

• Buffered formalin (light microscope preparation)

• Buffered gluteraldehyde (electron microscope preparation)

• Osmium tetroxide (electron microscope preparation, preserve and stain)

• Zenker's formal saline

• Bowen's fluid

No fixative will penetrate a piece of tissue thicker than 1 cm

Tissue is sectioned and drop-fixed in a 10% formalin solution (24-48 hours).Formalin has an

acidic nature. Prolonged fixation of samples in formalin causes formalin pigments inside the tissue. This pigments removed by washing sections with alcohol picric acid for some minutes

The excess of formalin can be removed by

Washing samples in running tap water (24 hr.)

Washing samples in 2 changes of chloral hydrate (12 hr. for each) .

The reactions of formalin with tissue are complex because it can combine with a different groups.

Tissue Processing

In order to cut thin sections of the tissues, it should have suitable hardness and consistency when presented to the knife edge.

These properties can be imparted by infiltrating and surrounding the tissue with paraffin

wax, various types of resins or by freezing.

Tissue Processing It can be subdivided into:

a) Dehydration

b) Clearing

c) Impregnation (infiltration)

Types of tissue processing

• There are two types:

1. Manual Tissue Processing

2. Mechanical Tissue Processing.

Manual Tissue Processing

• In this process the tissue is changed from one container of reagent to another by hand

Mechanical Tissue Processing

• In this the tissue is moved from one jar to another by mechanical device

• Timings are controlled by a timer which can be adjusted in respect to hours and minutes|

• Temperature is maintained around 60 ºC .

Dehydration (removal of water) :

It is the process in which the water content in the tissue to be processed is completely

removed by passing the tissue through increasing concentrations of dehydrating agents

Tissues are dehydrated by using increasing strength of alcohol; e.g. 70% (1 hours),80%(1

hours), 90% (1 hours),95% (30 minutes) and 100% (30 minutes).

• Water is replaced by diffusion

During dehydration water in tissue has been replaced by alcohol • the next step alcohol

should be replaced by paraffin wax • as paraffin wax is not alcohol soluble, we replace

alcohol with a substance in which wax is soluble. This step is called clearing.

When samples are dehydrated by Butyl alcohol, the clearing process can be neglected.

Clearing:

• Replacing the dehydrating fluid with a fluid that is totally miscible with both the

dehydrating fluid (alcohol) and the embedding medium (wax). Clearing process is a median

stage between Alcohol and paraffin wax.

Some clearing agents: - Xylene - Toluene - Chloroform – Benzene- Methyl benzoate.

Samples are cleared in 3 changes of Methyl benzoate (24 hr. for each change).

Infiltration (Impregnation):

The tissue is kept in a wax bath containing molten paraffin wax.

Samples are infiltrated in 3 changes of paraffin wax (1 hr for each change).

Embedding:

Embedding: is the process by which impregnated tissues are surrounded by a medium such as

agar, gelatin, or wax which when solidified will provide sufficient external support during

sectioning:

Embedding: It is done by transferring the tissue to a mould filled with molten wax & is

allowed to cool & solidify After solidification, a wax block is obtained which is then

sectioned to obtain ribbons

General Embedding Procedure.

1-Fill the mould with paraffin wax

2-Using warm forceps select the tissue, take care that it does not cool in the air

3-Orienting the tissue in the mould

Orientation Of Tissue In The Block

Correct orientation of tissue in a mould is the most important step in embedding

1. cross section

2. longitudinal section

4-Cool the block on the cold plate.

Sectioning (Section Cutting)

It is the procedure in which the blocks which have been prepared are cut or sectioned and thin

strips of uniform thickness are prepared

The instrument by which this is done is called as a Microtome.

TYPES OF MICROTOMES:

- Rotary microtome
- Freezing (cryostat) microtome
- Ultra microtome

Rotary Microtome
Freezing (Cryostat) microtome

4-Sectioning: Ribbon of sections

Tissue floatation bath

Taking the floating sections onto slide

Adhesives used for fixing the sections on the slides
Albumin solution (Mayor's egg albumin)

It is a thermostatically controlled water bath

It is maintained at a temperature 5 – 6 degrees below the melting point of paraffin wax.

Flattened paraffin sections. Taking the floating sections onto slide. Adhesives used for fixing

the sections on the slides. Albumin solution (Mayor's egg albumin).
*Flattened paraffin sections

Taking the floating sections onto slide

Wax preparation can't be used to demonstrate lipids because

1- Samples are exposed to high temperature.

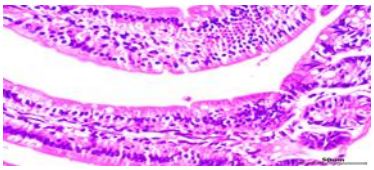
2- Lipid-soluble materials are used during preparation .

So, frozen preparation is a good method for demonstration of lipids and enzymes.

Under the frozen temperature, the enzymes are broken.

Frozen microtome and cryostat is used to cut the frozen samples.

1-Hematoxylin and Eosin



Ionic bonding is the most important type of bonding that occurs in histologic staining

techniques. It involves electrostatic attraction between ions of opposite charge, one of which

is fixed in the tissue, and the second of which is in the dye.

Hematoxylins alone is not technically a dye, and will not directly stain tissues. It therefore

needs to be used in combination with a “mordant” – a compound that helps it link to the

tissue. The mordant used is typically a metal cation, such as aluminium. Haematoxylin in

complex with aluminium salts is cationic and acts as a basic dye. It is positively charged and

can react with negatively charged, basophilic cell components, such as nucleic acids in the

nucleus. These stain blue as a result.

Eosin is anionic and acts as an acidic dye. It is negatively charged and can react with

positively charged, acidophilic components in the tissue, such as amino groups in proteins in

the cytoplasm.

Hematoxylins & eosin staining depends on the basic and acidic properties of tissue

components.

Hematoxylene is a basic dye, acidophilic dye

Eosin is acidic dye, basophilic dye.

The staining procedure for H&E follows a basic protocol:

- Dewaxing
- Dehydration
- Hematoxylin
- Differentiation
- Bluing
- Eosin
- Dehydration
- Clearing
- Cover-slipping

H & E Stain

Intended use

H & E Staining method is used for the routine staining of the cationic and anionic tissue components in tissue sections. This is the standard reference stain used in the study of histochemical tissue pathology.

Summary

Hematoxylin and eosin stain (abbreviated as H & E stain) is one of the principal tissue stains used in routine histology staining methods. It is the most widely used stain in medical diagnosis and is often the gold standard; wherein, when a pathologist looks at a biopsy of a suspected cancer, the histological section is likely to be stained with H & E.

H & E Stain is the combination of two histological stains: Hematoxylin and Eosin. The hematoxylin is a selective nuclear stain which stains the cell nuclei blue, and eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combinations of these colors. The stain shows the general layout and distribution of cells and provides a general overview of a tissue sample's structure. Hence, there is a clear differentiation between the nuclear and cytoplasmic parts of a cell.

Principle

Hematoxylin and eosin are the principle stains used for the demonstration of nucleus and the cytoplasmic inclusions. Alum acts as a mordant and hematoxylin containing alum stains nucleus light blue which turns red in the presence of acid. The cell differentiation is achieved by treating the tissue with acid solution. Counter staining is performed by using eosin solution which imparts pink color to the cytoplasm.

Hematoxylin, a common nuclear stain, is isolated from an extract of logwood (Hematoxylins campechianum). Before hematoxylin can be used as a nuclear stain, it must be oxidized to hematein and combined with a metallic ion (mordant). Most successful mordants have been salts of aluminum or iron. Generally, hematoxylins are classified as progressive or regressive based on dye concentration. Progressive stains (e.g., Mayer’s hematoxylin) have a lower concentration of dye and selectively stain

nuclear chromatin. The desired intensity is a function of time. Regressive stains (e.g., Harris hematoxylin) color all nuclear and cytoplasmic structures intensely. To arrive at correct chromatic response, excess dye must be removed by treatment with dilute acid (differentiation).

Eosin is tetra bromofluorescein (a substituted xanthene), a red acidic dye and fluorochrome. The dye is very soluble in ethyl alcohol and also used for the staining of cytoplasm. Eosin Y is the most commonly used counterstain for hematoxylin.

Reagents / Contents

1. Hematoxylin Harris

Hematoxylin 5.0 g

Ammonium/ Potassium Alum 100 g

Mercuric Oxide 2.5 g

Alcohol 95% 50 mL

Distilled Water 1000 mL

Appearance: Maroon purplish solution.

2. Eosin (AQU.) 2%

Eosin-Y 2.0 g

Distilled water 100 mL

Appearance: Dark reddish solution.

Storage and Stability

Store at 15°C-25°C away from bright light. Use before expiry date on label.

Materials required but not provided

Tissue section specimen on clean grease-free glass slide, staining rack, blotting paper, immersion oil, and microscope. Reagents and solutions required but not provided in the Kit such as xylene, a series of descending

and of ascending grades of alcohol, 1% acid alcohol solution, Scott's Tap Water Buffer (Cat. No. 207191390035) and DPX mountant.

Type of Specimen

Histochemical tissues sections obtained from biopsy specimens.

Procedure

1. Sections are deparaffinized (removal of wax) by placing in xylene for 10 - 15 minutes.

2. Rehydrate section by passing in a series of descending grades of alcohol, finally to water.

3. Place in Hematoxylin Harris solution for 8-10 minutes.

4. Rinse in water.

5. Differentiate the slide in a solution 1% acid alcohol for 10 seconds.

6. Rinse in tap water.

7. Blueing (brining the required blue color to section) is done by putting the section in a solution containing Sodium bicarbonate, $MgSO_4$ and saturated solution of Lithium carbonate (Scott’s Tap Water Buffer, Cat. No. 207191390035) for 2-10 minutes.

8. Counter stain with aqueous Eosin (Aqu.) 2% for 1-3 minutes.

9. Rinse in tap water.

10. Section are dehydrated which is done by a series of ascending grades of alcohol and finally clearing in Xylene.

11. Dry the section by pressing on the filter paper.

12. Mount in DPX and observe under microscope, 40X and 100X under oil immersion lens.

Interpretation of Results

The nuclei of cells are stained blue or dark-purple along with a few other tissues, such as keratohyalin granules and calcified material with Hematoxylin. The cytoplasm and some other structures including extracellular matrix such as collagen stains in up to five shades of pink with Eosin. Most of the cytoplasm is eosinophilic and is rendered pink. Red blood cells are stained intensely red. The background of the tissue remains colorless.

Warranty

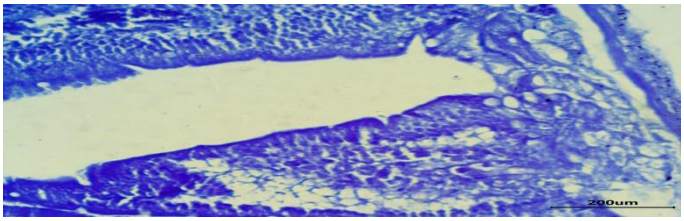
H & E staining solutions are for “In Vitro Diagnostic Use” only. This product is designed to perform as described on the label and pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

https://l.facebook.com/l.php?u=https%3A%2F%2Fyoutu.be%2FsHeHC_0-BDw%3Ffbclid%3DIwAR0pdzfMyxLOKbdwWpXSlG5In7cN3tDLgx4vxK2Go8_k1vZcYgKzWiaN72s&h=AT08dbg55gJ5eM7tl5nWDgEuk6AYzntHakmYxOmTPochzPV_PB7kOLkjClXzwxwWX1MM4uUc2bkKc7u1y-zvRo_KRoB_r_hZ2QHvG_IarmP7m-EIXGmUm0iCbzXqt2DkP1ZE

2- Demonstration of proteins (Mercury_ Bromophenol blue method):



Proteins are complex, organic compounds composed of many amino acids linked together

through peptide bonds and cross-linked between chains by sulfhydryl bonds, hydrogen bonds

and van der Waals forces.. The proteins in the various animal and plant cells confer on these

tissues their biological specificity.

One of the most important characteristics of the aminoacids in their capacity of combining

with each other to form long chains. This property is due to the presence of the carboxyl

group (-COOH) and the basic amino group (-NH2).

Proteins are amphoteric because it contains the carboxyl group (-COOH) and the basic amino

group (-NH2).

Mechanism of dye

1- Dye deposition

2- Add a weak acid

3- pH<3

4- Protein behaves like a base

The color density of Bromophenol blue stain depends on amount of proteins in tissue

parts.

In some cases, Bromo-phenol stain give red color because it Metachromatic +

Dichromatic stain.

Principle

Mercuric ions of the bromophenol blue solution react with

acidic, sulphydryl and aromatic residues of the protein to give

blue colour.

Fixation and Section

10% neutral buffered formalin; paraffin.

Reagent

Mercuric bromophenol blue : Dissolve 1 gm mercuric chloride

and 0.05 gm. bromophenol blue in 100 ml 2% aqueous

acetic acid.

Method

Bring sections to water. Stain in the mercuric bromophenol

blu2 solution for 2 hours at room temperatue. Rinse sections for

5 minutes in 0.5% acetic acid. Transfer sections dirctly into

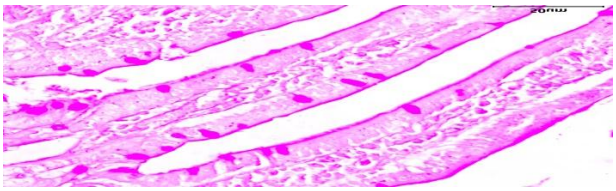
tertiary butyl alcohol. Clear in xylene and mount in DPX.

Result

Proteins—deep blue color

https://l.facebook.com/l.php?u=https%3A%2F%2Fyoutu.be%2FroWDBmUDLUg%3Ffbclid%3DIwAR292x50HyfbE0QaxdN3WAGjQ15KW5aiLzYZBU5jM-5TTot3nm3Sj86kHZc&h=AT08dbg55gJ5eM7tl5nWDgEuk6AYzntHakmYxOmTPochzPV_PB7kOLkjClXzwxwWX1MM4uUc2bkKc7u1y-zvRo_KRoB_r_hZ2QHvG_IarmP7m-EIXGmUm0iCbzXqt2DkP1ZE

Carbohydrates:



Carbohydrates are keto-derivatives or aldehyde –derivatives. Carbohydrates may be

classified as mono saccharides, disaccharides , polysaccharides.

1- Mono saccharides are simple sugars which cannot be hydrolyzed into a simpler form .

2- Disaccharides are the result of condensation of two molecules of mono saccharides

with the loss of one molecule of water.

Maltose is built up of two molecules of glucose. Lactose is built up of glucose and

Gala ctose. Sucrose is formed of glucose and fructose.

3- Poly saccharides are formed by the condensation of many molecules of

Mono saccharides with a corresponding loss of water molcules.

Glycogen is the simple polysaccharide of the animal body. It is often called animal starch.

It is contained in liver cells and muscle fibers.

Sometimes, glycogen is not uniformly distributed in the cytoplasm of fixed cells

particularly in the liver cells where it appears accumulated in certain regions of the cells,

whereas the other regions remain almost empty. This phenomenon is referred to as"

glycogen flight". It is explained as being due to the fact that the fixatives used for the

preservation of glycogen sweep this materials infront of them until it becomes pressed

against the part of the cell membrane opposite to the direction of diffusion.

Glycogen in the liver cells

Liver glycogen has two main source. The first is the glucose carried from alimentary

canal. The second source is the lactic acid produced in the muscle cells as a result of

glycolysis.

To avoid glycogen flight

1- Using frozen preparation method

2- Wash samples after fixation in diluted Osmic acid (2 min).

Description: This method is used for detection of glycogen in tissues such as liver, cardiac and skeletal muscle on formalin-fixed, paraffin-embedded tissue sections, and may be used for frozen sections as well. The glycogen, mucin, and fungi will be stained purple and the nuclei will be stained blue.

Fixation: 10% formalin.

Section: paraffin sections at 5 um.

Solutions and Reagents:

0.5% Periodic Acid Solution:

Periodic acid ---------- 0.5 g

Distilled water ---------- 100 ml

Schiff Reagent: Test for Schiff reagent: Pour 10 ml of 37% formalin into a watch glass. To this add a few drops of the Schiff reagent to be tested. A good Schiff reagent will rapidly turn a red-purple color. A deteriorating schiff reagent will give a delayed reaction and the color produced will be a deep blue-purple.

Mayer's Hematoxylin Solution:

Procedure:

1. Deparaffinize and hydrate to water.
2. Oxidize in 0.5% periodic acid solution for 5 minutes.
3. Rinse in distilled water.
4. Place in Schiff reagent for 15 minutes (Sections become light pink color during this step).
5. Wash in lukewarm tap water for 5 minutes (Immediately sections turn dark pink color).
6. Counterstain in Mayer's hematoxylin for 1 minute.
7. Wash in tap water for 5 minutes.
8. Dehydrate and coverslip using a synthetic mounting medium.

Results:

Glycogen, mucin and some basement membranes --- red/purple

Fungi ---------- red/purple

Background ---------- blue

Demonstration of glycogen (Best's carmine method):

The reason is that complex sugars include a high amount of hydroxyl groups, which gives the

complex sugar molecule an anionic nature (negative ion). As for the dye in all media, it

behaves like a positive ion, and tends to combine strongly with complex sugars by forming strong hydrogen bonds.

Stain procedure.

1 – Digest sections (see above)

2 – Stain in hematoxylin for 10 mins to stain nuclei.

3 – Rinse rapidly in 1% acid alcohol to clear the background.

4 – Wash well in tap water to remove acid alcohol.

5 – Stain in Best's Carmine stain for 10-15 minutes ina Coplin jar.

6 - Wash in Differentiator solution until red stain stops running from the slide (usually takes just a few seconds).

7 - Dehydrate through alcohols and xylene and mount coverslip in DPX.

8- Examine.

Results:

Glycogen Red

Nuclei Blue

https://www.youtube.com/watch?v=QDN0evB5Ca8

Nucleic acids(Feulgen reaction):

PRINCIPLE :

Acid hydrolysis, designed to separate selectively 2 purine bases, namely adenine and guanin, from DNA molecule;staining of apurinic acid resulting from hydrolysis with Schiff reagent. This reagent can be used since free deoxyribose changes to aldehyde in acid environment. Feulgen reaction is highly selective for DNA. Moreover, this reaction allows a very precise localisation of DNA .

PROCEDURE :

1. Bring section to distilled water Put on the section 10 drops of R1, leave to act 10 minutes.

Double washing in distilled water

2. Put on the section 10 drops of R2, leave to act 10 minutes .

3. Drain the slides without washing and put on the section 10 drops of R3 , leave to act 2 minutes .

Wash in runing tap water for 5 min .

4. Counterstain, if required, put on section 10 drops of reagent (4) for

two minutes, then wash with distilled

water .

Dehydrate through ascending alcohols;

clear in xylene and mount

RESULTES :

Nuclear DN : appears reddish purple

Cytoplasm : green .

REFERENCE :

De Tomasi JA. Improving technique of the

Feulgen stain . Stain Technol 1936; 11: 37 .

https://www.youtube.com/watch?v=iSCrTSZotjM

Red blood cells count method

The red blood cells (RBCs) count method comes under haemocytometry, which quantitatively measures the number of RBCs in a blood sample. Haemocytometer slide is a manual method to count RBCs.

Principle of red cell count

It is not possible to directly count the RBCs in a blood sample. Thus, it is necessary to dilute the blood sample or blood specimen using one of the RBC diluting fluids (Nacl 0.9% is isotonic solution for RBCs cells).

What is RBC?

RBCs stand for red blood cells. It is also called erythrocytes, which appears red-coloured due to the coloured pigment (**haem**) and exists as a biconcave disc. RBCs possess a diameter of 7.5 to 8.7 μm and a thickness of 1.7 to 2.2 μm. It lacks a nucleus and has a life span of 120 days.

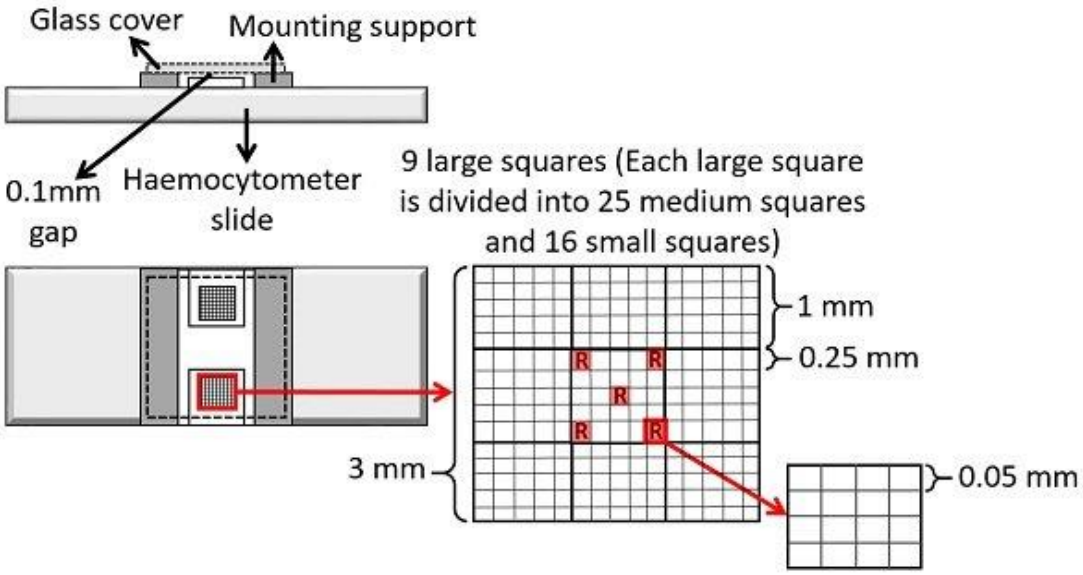
Requirements of Total RBC Count :

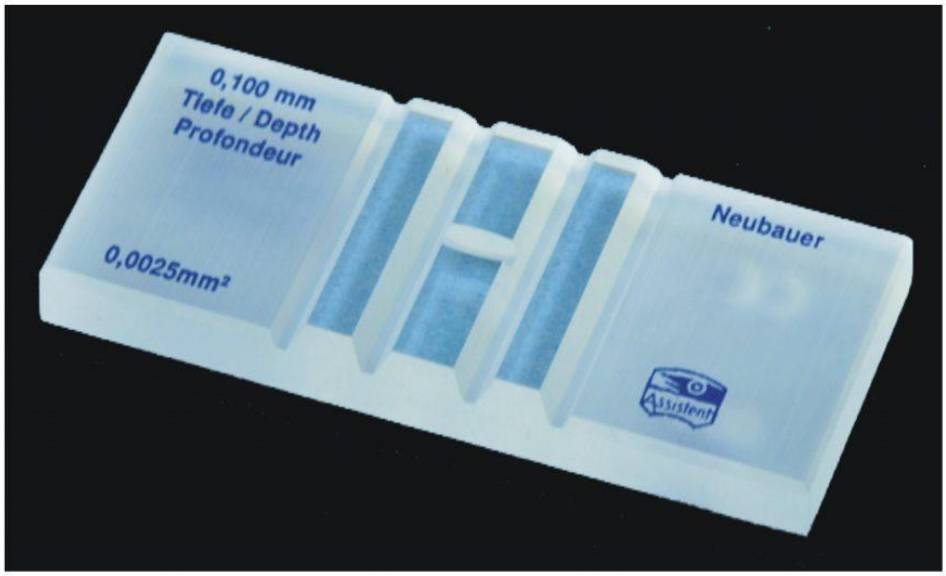
Haemocytometer refers to the micro-slide through which the number of erythrocytes or RBCs can be enumerated.

Haemocytometer :

It is a specialized thick glass slide used to count the eukaryotic cell suspension. Haemocytometer has a size of 30 X 70 X 4 mm. Its central portion is ruled, where the cell counting is performed. The counting grid has a size of 3 mm X 3 mm. One can estimate the number of red blood cells using a haemocytometer after diluting the blood sample with RBC diluent.

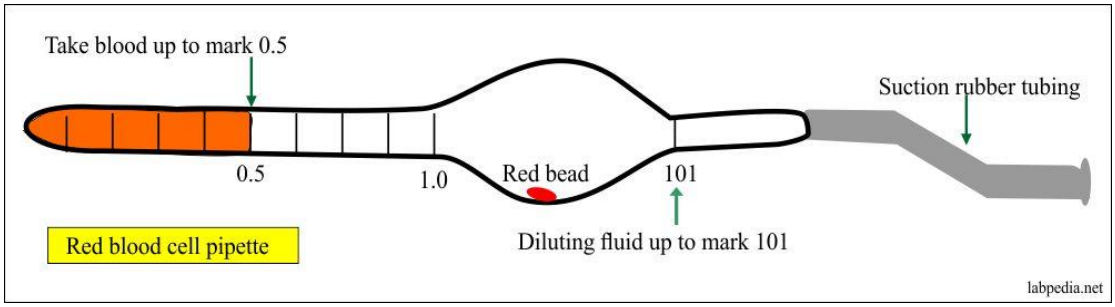
Neubauer's Chamber or Haemocytometer





Procedure:

1. RBCs counting solution is Nacl 0.9% isotonic saline.
2. Make a dilution of 1:200 with a diluting solution. Fill the red bulb pipette up to 0.5 marks.
3. Draw the solution to mark 101 of the RBC pipette.



4-Mix the blood thoroughly in the pipette.

1.Discard the first few drops (4 to 5) and then fill Haemocytometer.

.2Make sure that the chamber is free of air bubbles.

3.The distribution of the cells should be uniform over the ruled area.

5-Allow for 2 minutes to settle the cells.

6-Now count RBCs in the Haemocytometer.

1.Use 10 or 40 X to count the RBCs.

For RBCs, use the center square, which has 25 smaller squares.

7-Count the corner 4 squares and one central square.

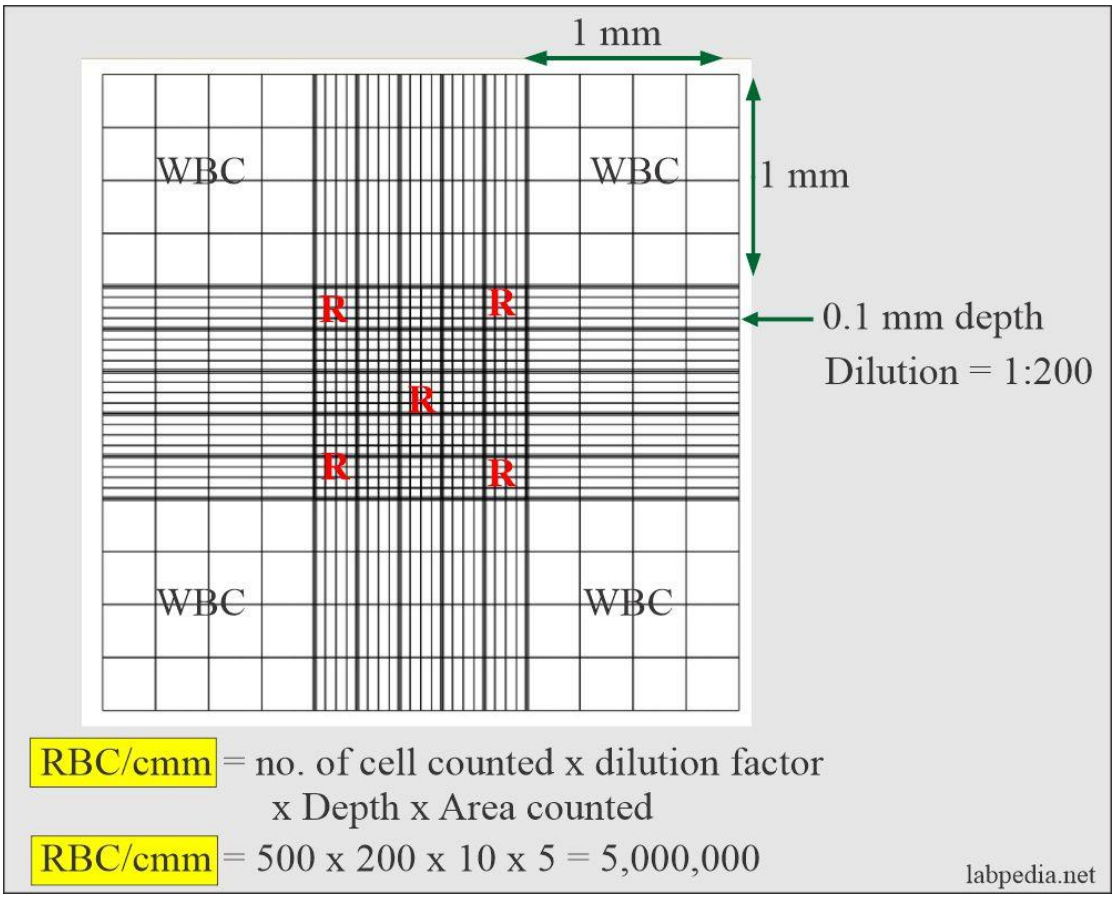
8-Count only the RBCs which fall on the left and top border of these squares.

9-Repeat the count twice and divide by 2 to get the average.

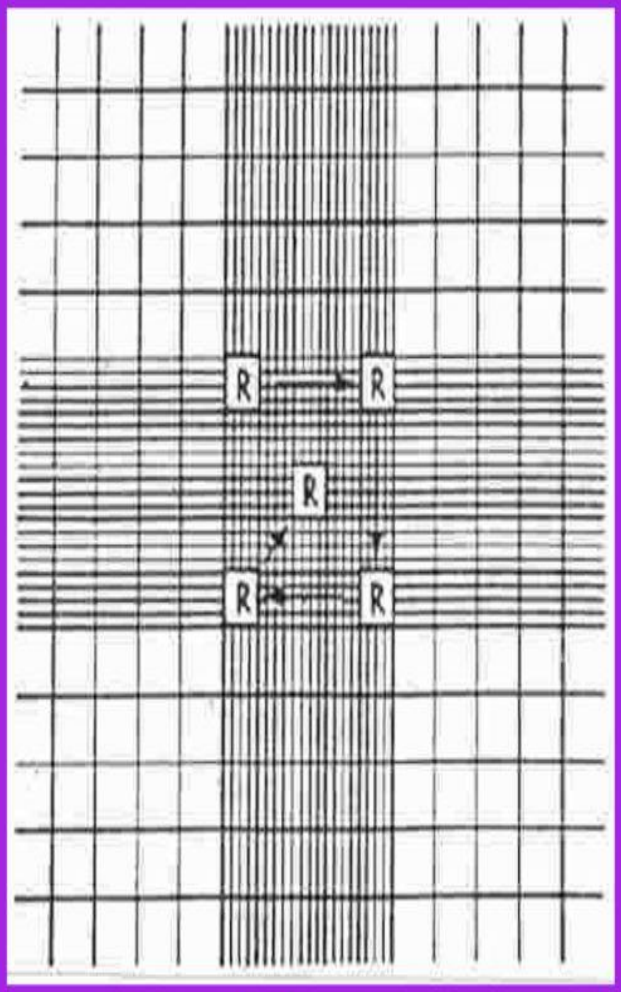
The formula for RBCs count is:

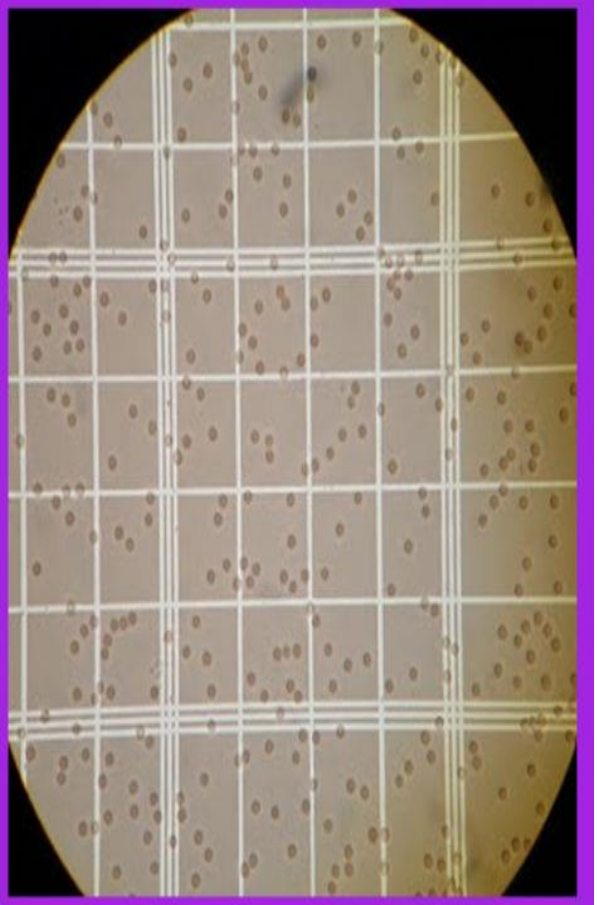
Multiply factor = 10 x 200 / 0.2 = 10,000

Multiply RBCs count with 10,000 = RBCs million/cmm.



Method determination of red blood cell (RBC) count





http://mbbsstudystuff.com/

White blood cells count method

The white blood cells also named leukocyte (WBCs) count method comes under haemocytometry, which quantitatively measures the number of WBCs in a blood sample. Haemocytometer slide is a manual method to count WBCs.

It is not possible to directly count the WBCs in a blood sample. Thus, it is necessary to dilute the blood sample or blood specimen using one of the WBC diluting fluids (acetic acid 0.2%).

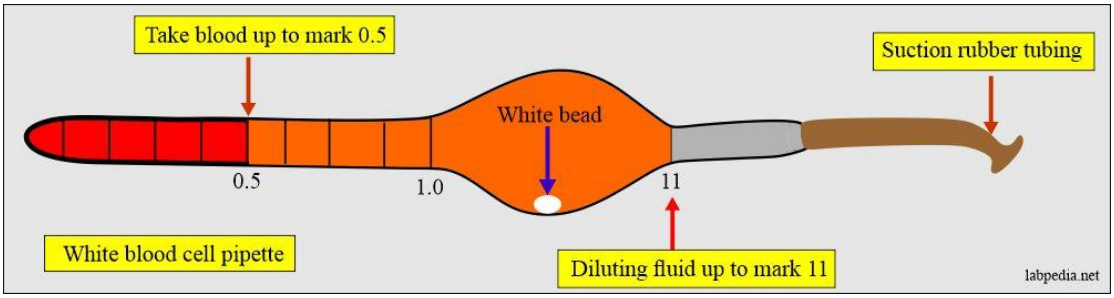
Principle of white cell count

Blood is diluted with a solution (acetic acid) that causes the lysis of RBCs. Acetic acid has no effect on the WBCs. Gentian violet is added to differentiate the WBCs.

1. Blood is diluted with a fluid that causes the RBCs' hemolysis, but WBCs remain intact, and then these are counted in the Neubauer chamber.
2. Gentian violet lightly stains the leucocytes and allowing those to be counted.

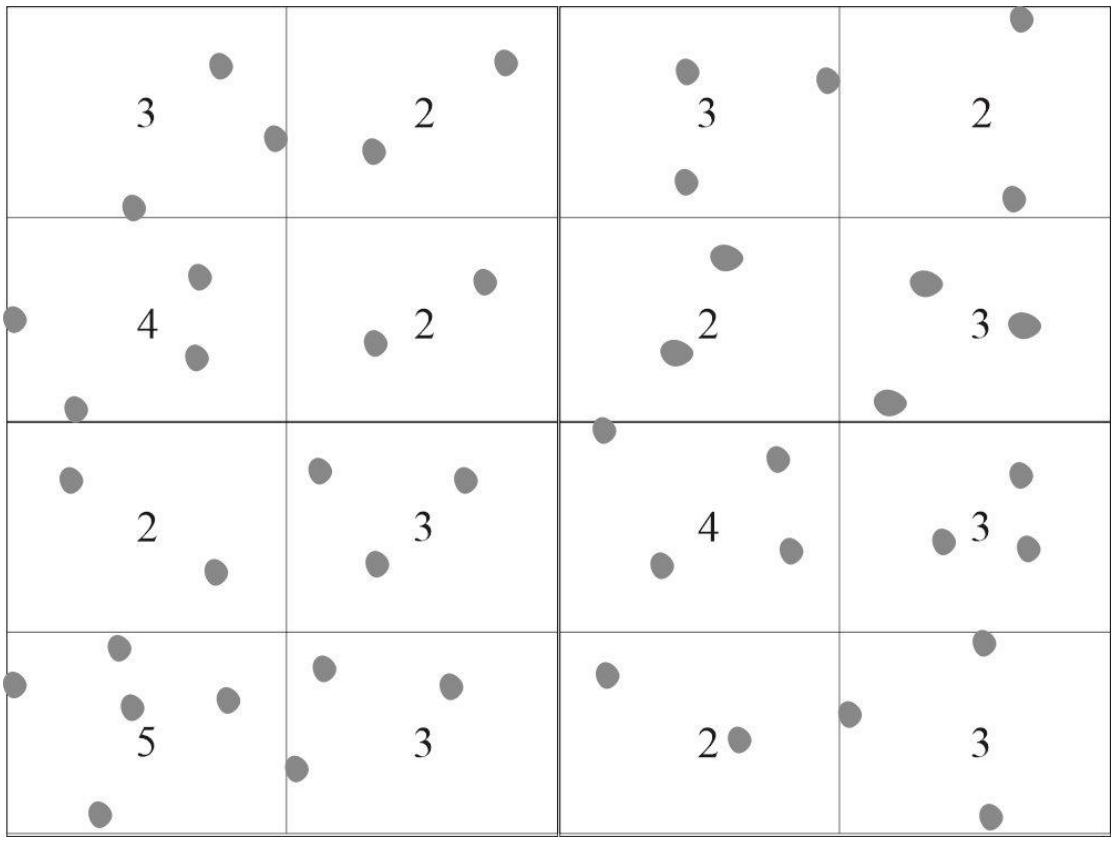
This pipette (also called Thoma pipette) long stem is divided into two parts:

1. The long stem is marked with 0.5 and 1.0
2. While the short arm after the bulb is marked 11.
3. Its central portion is a bulb or a globular shape with one white bead in it.
4. Rubber tubing is attached to suck the blood.
5. Ultimately the dilution of the blood to the TLC fluid is 1:20.



Procedure:

1. Take the WBC pipette, which has a white bead inside.
2. **WBC pipette method:**
3. Fill the blood into the 0.5 marks and then add the diluting solution.
    1. Fill the pipette with the diluting solution to point 11.
    2. Remove the rubber tubing.
    3. Seal both ends or hold in between two fingers.
    4. OR can put this pipette on the mechanical device to shake it.
    5. Shake for 1 minute or preferably for 2 minutes.
    6. After thorough mixing, discard the first few drops and then gently fill the chamber until the platform is filled.
    7. The capillary action will draw the fluid.
    8. Allow the chamber on the microscope stage for 2 to 3 minutes till the cells are settled.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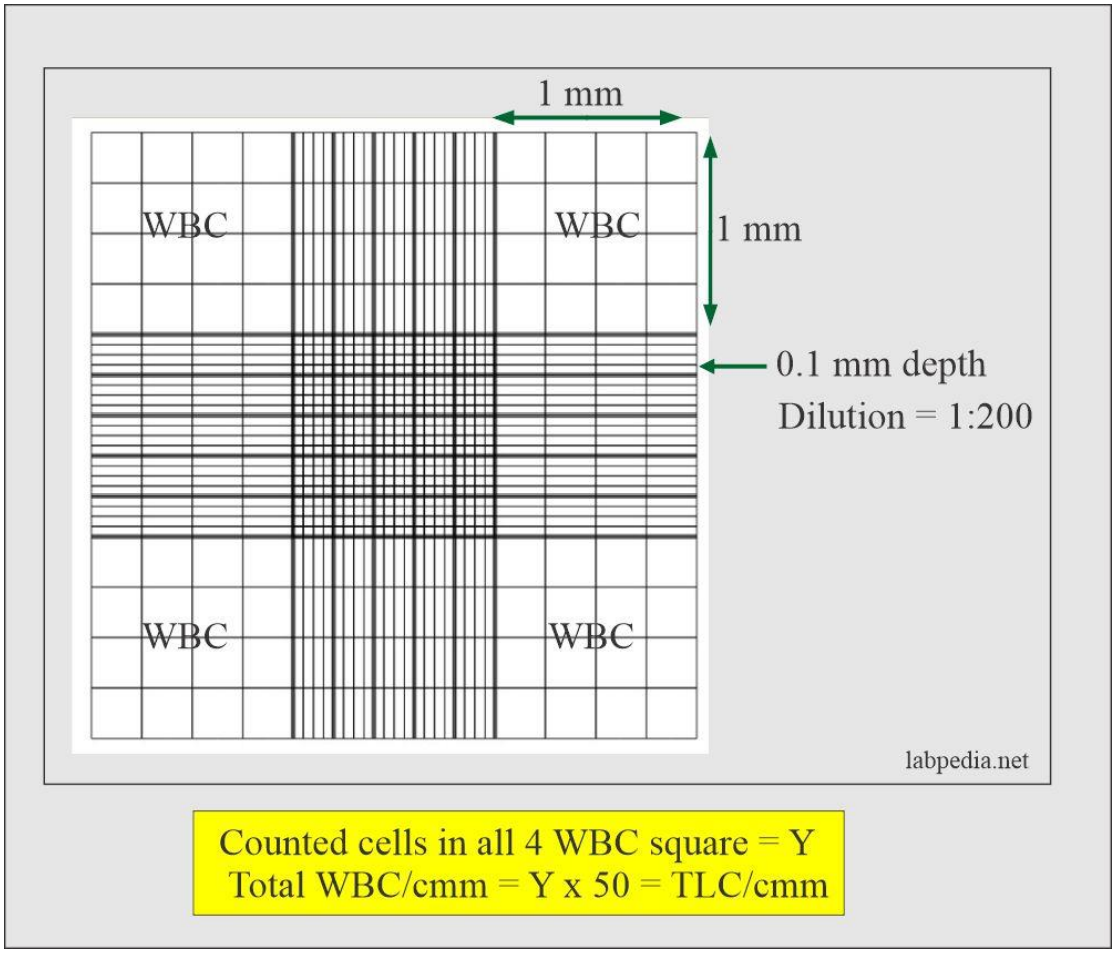
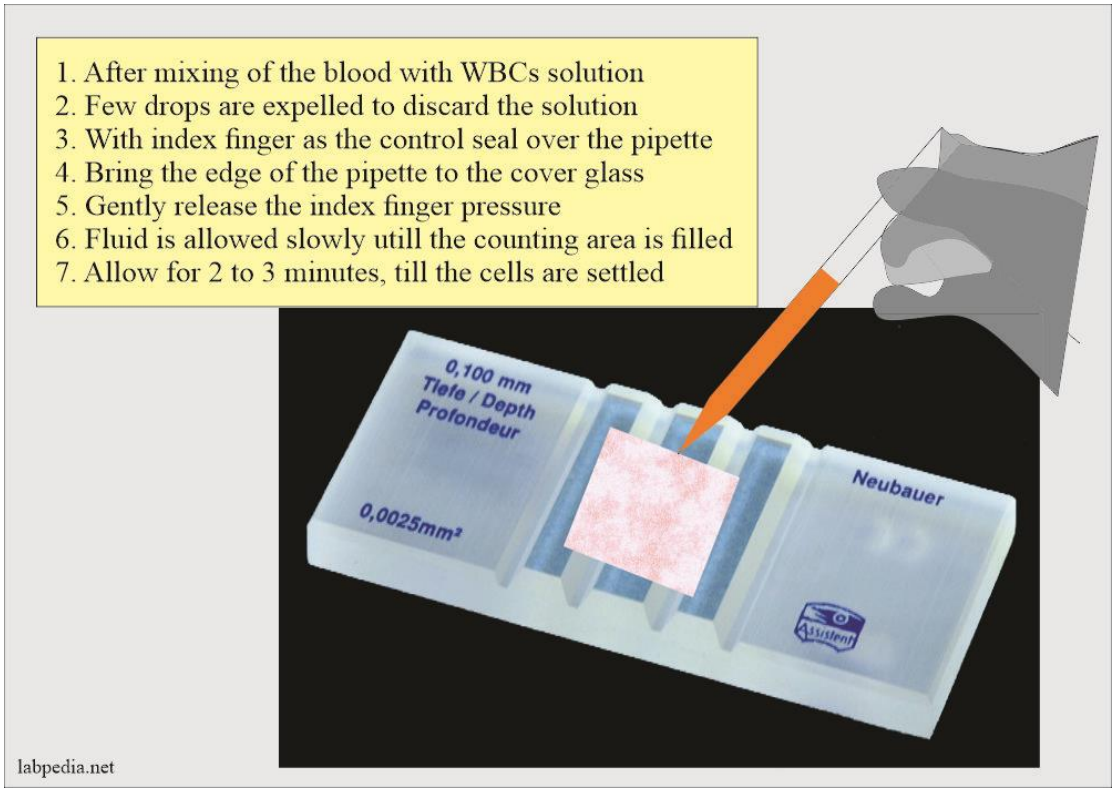

1 mm
1 mm
WBC
WBC
WBC
WBC
0.1 mm depth
Dilution = 1:200
labpedia.net
Counted cells in all 4 WBC square = Y
Total WBC/cmm = Y x 50 = TLC/cmm

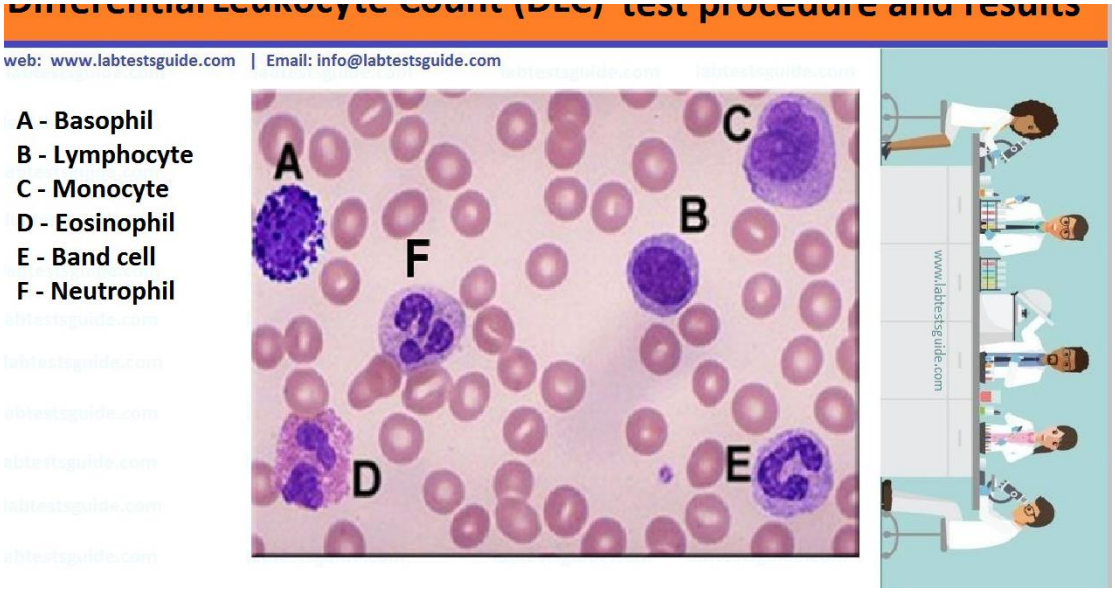


Calculations:

1. Count the cells in the haemocytometry. These are counted in the four large corner squares labeled as WBC and if the number is Y.
    1. One large area is 1 x 1 mm, and the depth is 0.1 mm.
    2. Total area counted in 4 large squares = 4 x 1 x o.1 = 0.4 µL (4/10).
    3. Y x 10/4 is the total WBC in the cell in 1 µL.
    4. Now dilution is 1:20.
    5. Number of WBC in 1µL = Y x 10 x 20/4 = Y x 50 = Total WBC count.
    6. **Total WBC = counted cells (Y) x 50 = WBC COUNT/cmm.**

Differential Leukocyte Count (DLC) Test

A differential blood count gives the relative percentage of each type of white blood cell and also helps to reveal abnormal white blood cell populations (eg,blasts, immature granulocytes, and circulating lymphoma cells in the peripheral blood).



have five types of white blood cells:

Neutrophils

Lymphocytes

Monocytes

Eosinophils

Basophils

Test Procedure

Leukocytes are classified into various groups depending on their size,

features of the nucleus and features of the cytoplasm. The WBCs exist in two forms viz. granulocytes and agranulocytes. Granulocytes are further

classified as eosinophil, basophil, neutrophil, while agranulocytes shows

lymphocytes and monocytes.

Sample Required:

1. The best sample is blood in EDTA.

2. Also, prepare fresh peripheral blood smear.

3. This is inexpensive, easy to perform and rapidly done as a screening test.

Test Requirements:

Well mixed whole or anticoagulent Blood

Glass Slides

Distilles Water

Stain (any one)

Leishman's stain

Wright Stain

Giemsa Stain

Field Stain

Dicrorizer (Any one)

Microscope

Preparing the Slide:

Collect drops of blood on the end side of a glass slide.

Spread the blood drop with another glass slide by placing it at an angle of 45 degree and move sidewise.

Hold the spreader firmly and move it on the previous slide to the other end ina straight line with same force and pressure.

Allow the glass slide to dry after formation of the smear.

Fix the smear with air dry or any other fixative/

Staining the Slide.

Observation the slide and counting of cells

Keep the prepared slide is under low power of microscope and choose a good quality slide.

Then identify different types of WBC under medium power.

Draw a table with 10 boxes both on horizontal and vertical axis on a observation notebook or use hand counter to count the cells.

Fix the slide on the plateform and choose a area towards the corner.

Count the different types of WBC found on the table in an abbreviated.

Move downwards and in chain like manner till 100 cells are observed.

After counting 100 cells prepare the report.

Hemoglobin determination

Hemoglobin (Hb or Hgb) is a red color pigment present in red blood cells (RBCs) comprises Fe2+ and Globin protein. It is Hemoglobin in RBCs that carries the oxygen from the lungs to the tissues and CO2 from body tissues to the lungs for excretion.

Hemoglobin (Hb or Hgb) is responsible for the appearance of Red color RBCs and blood. Hemoglobin is a chromoprotein consisting of Globin molecule attached to 4 red colored Heme molecules. Hemoglobin synthesis requires the coordinated production of Heme and Globin. Heme is a prosthetic group that medicates reversible binding of oxygen by hemoglobin. Globin is the protein that surrounds and protects the Heme molecule.

Sahli's Method Acid Hematin Method :

PRINCIPLE OF SAHLI's METHOD / ACID HEMATIN METHOD

The principle of Sahli's Method or Acid hematin method is quite easy that when the blood is added to 0.1% Hydrochloric acid (HCl), the hemoglobin present in RBCs is converted to acid hematin which is a dark brown colored compound. The color of the formed acid hematin complex corresponds to the Hemoglobin concentration in the blood and is matched with the standard which is a reference brown glass given in the Sahli's apparatus by diluting with 0.1% hydrochloric acid or distilled water until the color of acid hematin complex match with the color of the standard.

REAGENTS REQUIRED FOR SAHLI's METHOD / ACID HEMATIN METHOD

- 0.1% hydrochloric acid (It is prepared by diluting concentrated hydrochloric acid in distilled water and volume is made up 100 ml).
- Distilled water

APPARATUS & EQUIPMENTS REQUIRED FOR SAHLI's METHOD / ACID HEMATIN METHOD

- Sahli's Apparatus
    - Hemoglobin pipette (0.02 ml or 20 µl capacity)
    - Sahli's graduated Hemoglobin tube
    - Thin glass rod Stirrer for Hemoglobin Tube
    - Sahli's Comparator box with brown glass standard

PROCEDURE OF SAHLI's METHOD / ACID HEMATIN METHOD

0.1% Hydrochloric acid is taken in Hemoglobin tube (has two graduations – one side gm/dl, and other side shows the Hb %). ⇒

Dispense the blood into 0.1% hydrochloric acid taken in the hemoglobin tube, rinse the pipette with the same solution and mix properly with the help of stirrer.

⇒ Place the tube at room temperature for 5 minutes for complete conversion of hemoglobin into acid hematin.

⇒ After the reaction completes, place the Hb tube in the column in Sahli's Comparator box and start diluting the dark brown coloured compound (Acid Hematin) formed in the Hb tube using the N/10 HCl or distilled water by adding drop by drop of it into the solution and mix with the help of stirrer after each addition.

⇒ This process is done until the endpoint comes matching the color of standard with the color of the test.

⇒ Once the color is matched with the standard brown glass, lift the stirrer up and note down the reading in Sahli's Hb tube by taking the lower meniscus in consideration.

PRECAUTIONS TO BE TAKEN WHILE PERFORMING ESTIMATION OF HEMOGLOBIN BY SAHLI's METHOD / ACID HEMATIN METHOD:

⇒ Sahli's apparatus especially the Hemoglobin pipette and Sahli's Hemoglobin tube should be clean and dry before use.

⇒ Suck the blood exactly up to the mark of 20 µl (0.02 ml) and air bubbles should not be present in the pipette with blood.

⇒ Mix well the acid and blood and wait for at least 5 minutes after adding the blood in acid.

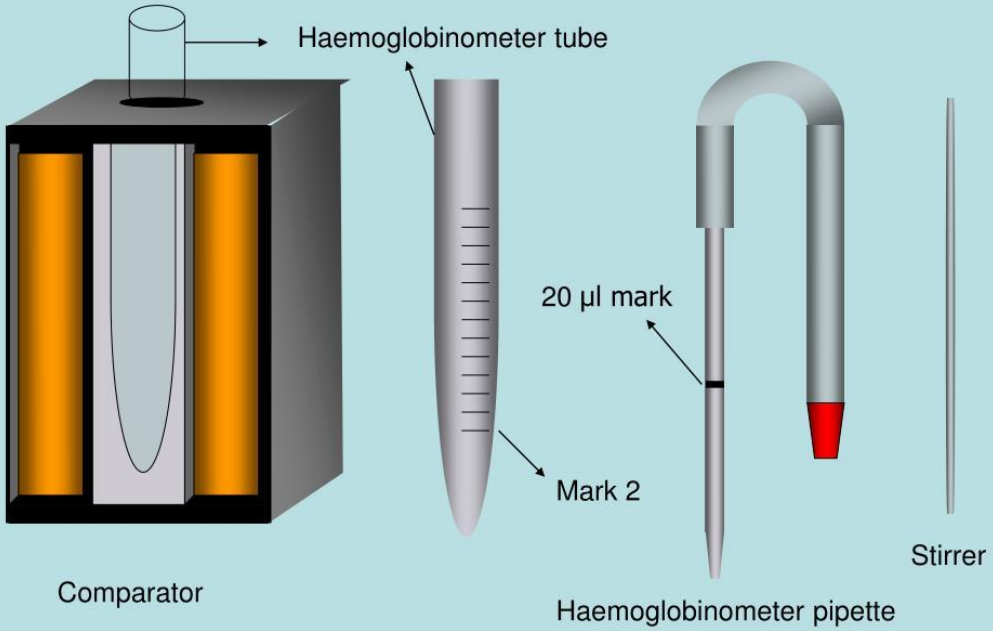
Add distilled water drop by drop and mix well after each dilution. Avoid over dilution of the content.

⇒ The matching of color should be done against the natural source of light or electrical tube light (white light) to avoid any visual errors.

⇒ Blood sample and 0.1% HCl acid should be taken in an accurate and precise amount in the Hb tube.

⇒ The Hb pipette should be wiped off properly in order to avoid the excess addition of blood in the Acid.

SAHLI'S HAEMOGLOBINOMETER



Determination of Hematocrit (Hct) (Packed Cell Volume; PCV)"

Hematocrit:Hematocrit is defined The percentage by volume of packed red blood cells in a given sample of blood after centrifugation.The hematocrit may also be referred to as Packed Cell Volume (PCV) or erythrocyte volume fraction (EVF).

Purpose for doing the Hct

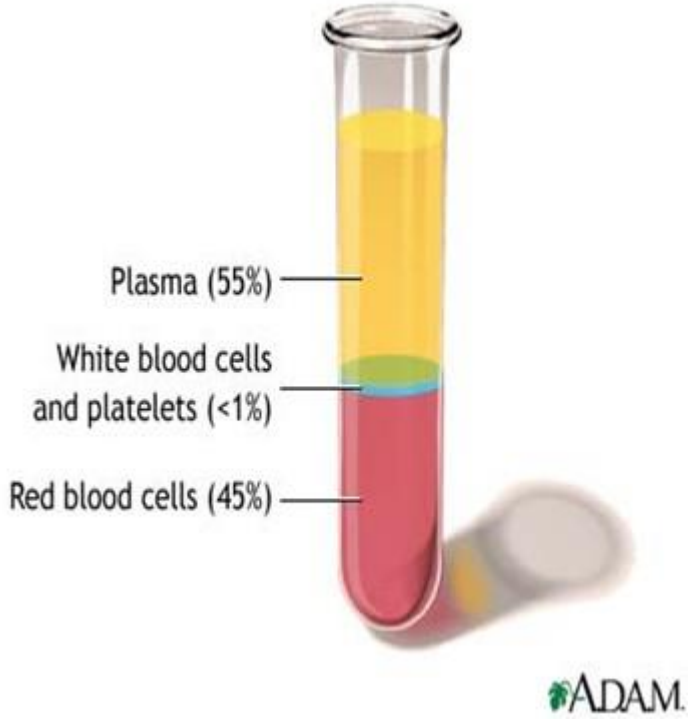
Blood is made up of red and white blood cells, platelets, and plasma.A decrease in the number or size of red cells also decreases the amount of space they occupy, resulting in a lower hematocrit.An increase in the number or size of red cells increases the amount of space they occupy, resulting in a higher hematocrit.

Procedure:

Take blood by capillary tube by capillary.

Close the hole that took blood , close it with wax,clay and soap.

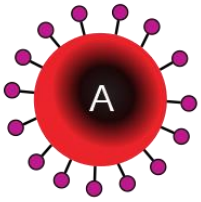
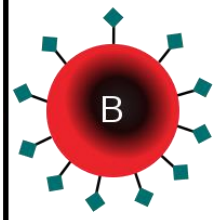
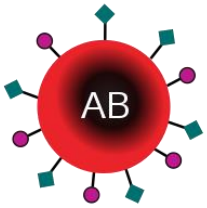
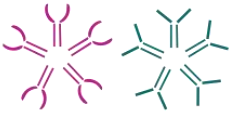
the tubes in a centrifuge (5 minutes at rpm),.7. Using a special reading device(since the capilary tube is not graduated).



ABO blood group system

The **ABO blood group system** is used to denote the presence of one, both, or neither of the A and B antigens on erythrocytes. In human blood transfusions it is the most important of the 38 different blood type (or group) classification systems currently recognized. A mismatch (very rare in modern medicine) in this, or any other serotype, can cause a potentially fatal adverse reaction after a transfusion, or an unwanted immune response to an organ transplant. The associated anti-A and anti-B antibodies are usually IgM antibodies, produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses.

The ABO blood types were discovered by Karl Landsteiner in 1901; he received the Nobel Prize in Physiology or Medicine in 1930 for this discovery.[4] ABO blood types are also present in other primates such as apes and Old World monkeys.

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in red blood cell	A antigen	B antigen	 A and B antigens	None

Coagulation Time

Coagulation Time (Clotting Time) CT.

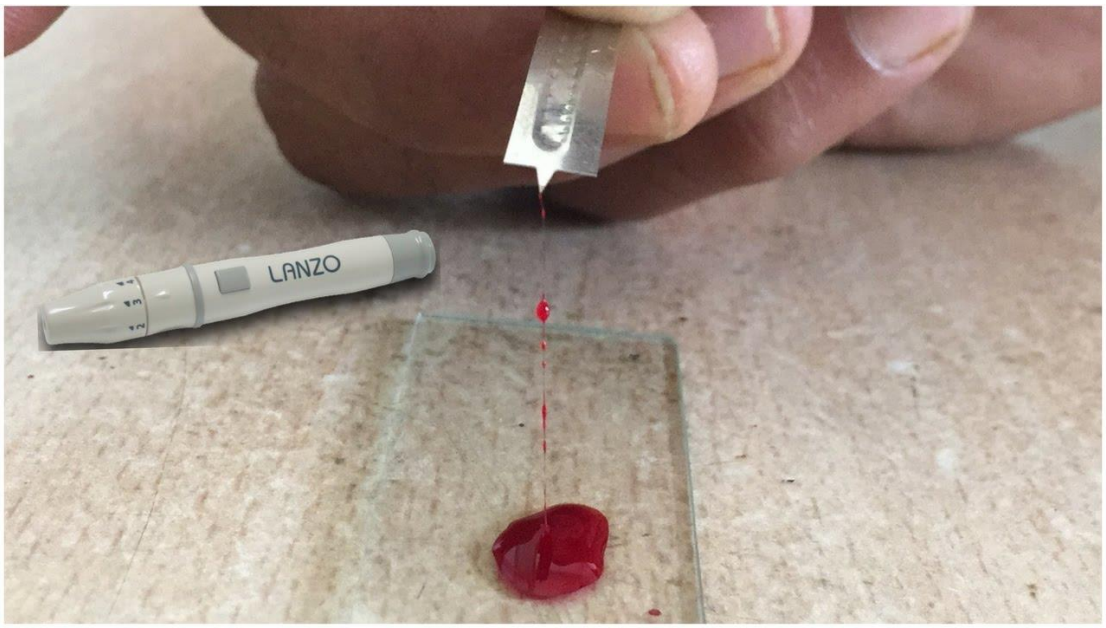
CT: the of whole blood is length of time required for a measured amount of blood to clot under certain specified conditions (the time required for blood to form aclot).

Slide methode

Procedure:

Place 1 ml from blood on slide then each 30 seconds check blood even formation threads .

CLOT FORMATION

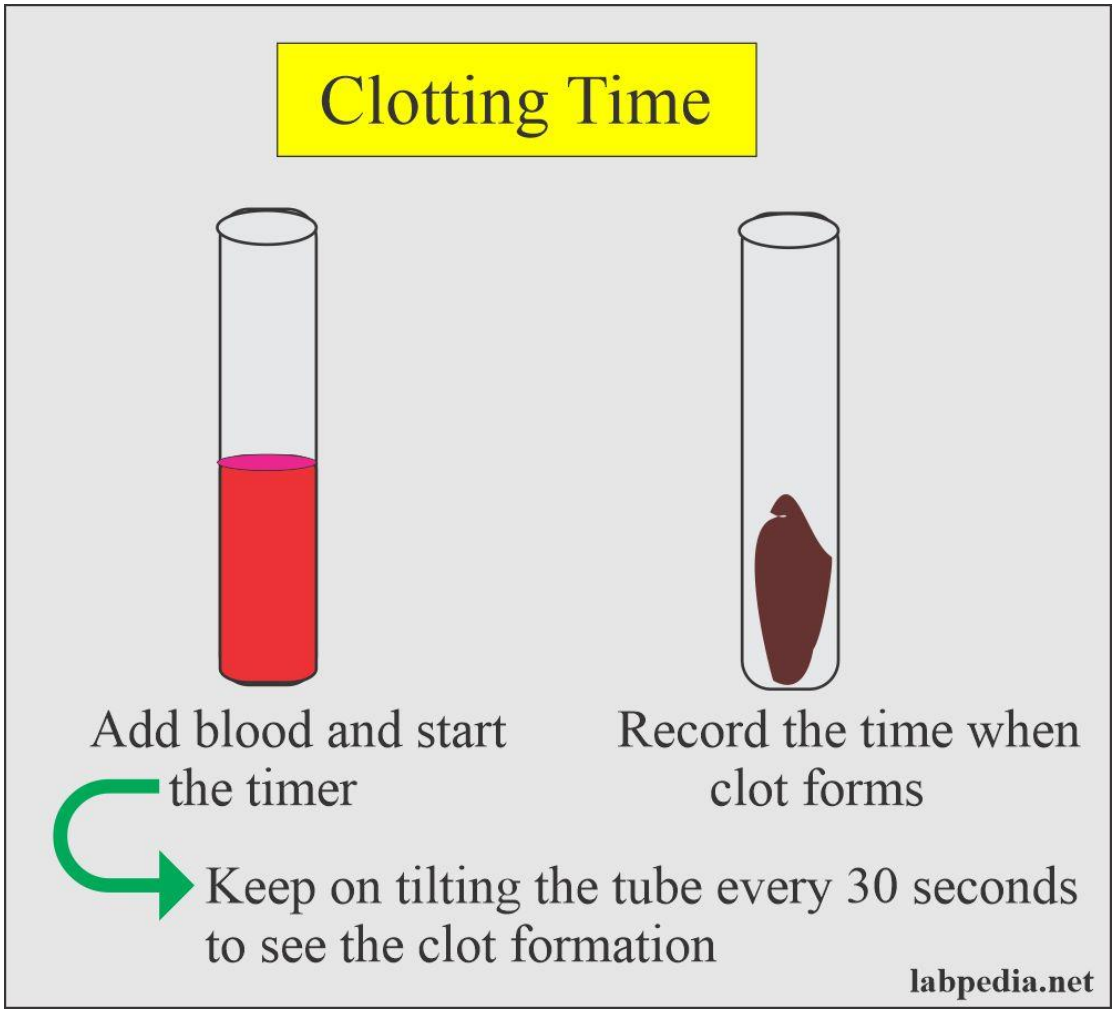

LANZO

test tube method:

1. Perform this test at 37 ° C.
2. For the tube method, take 4 ml of blood and start the time.

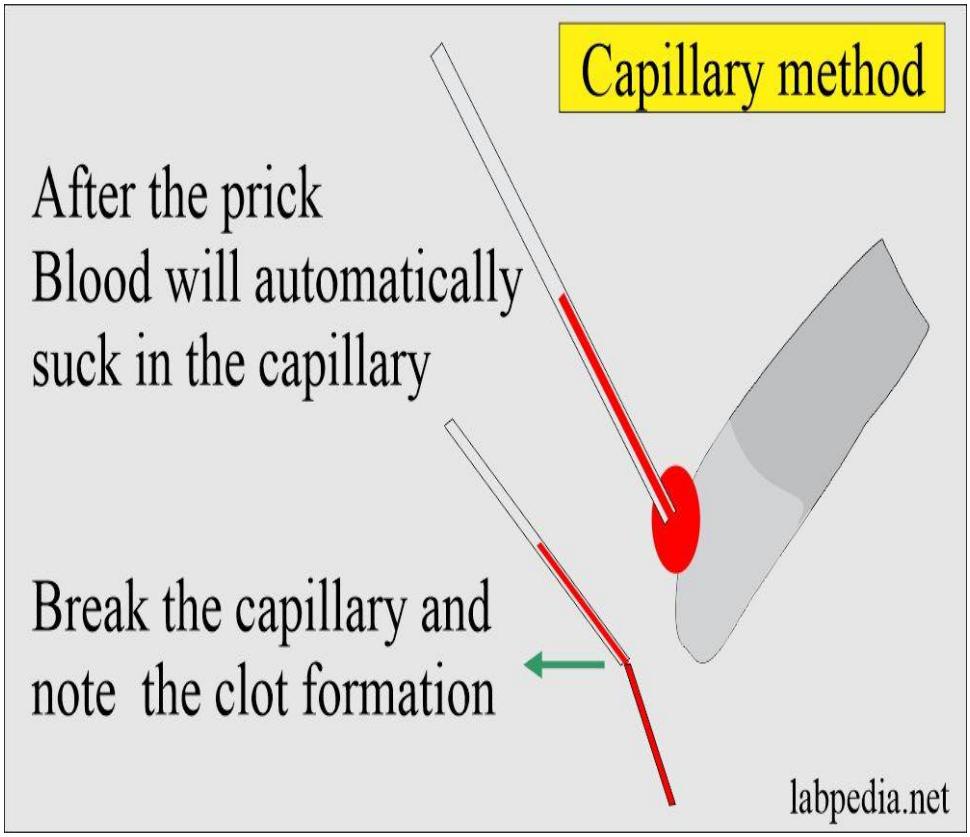
3.Note the time when there is the first appearance of the clot formation.

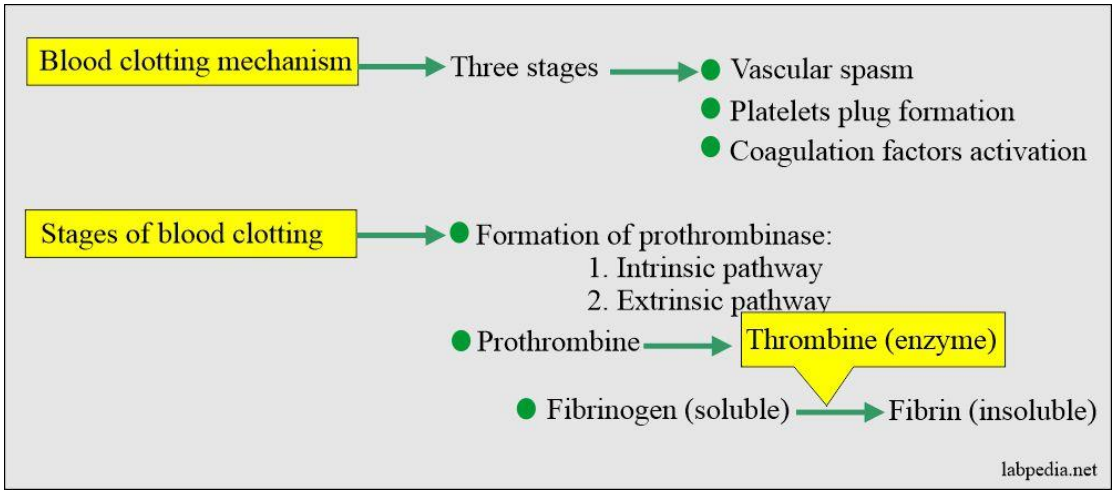
4.This test can be done in multiple tubes to be more accurate.



Capillary method.

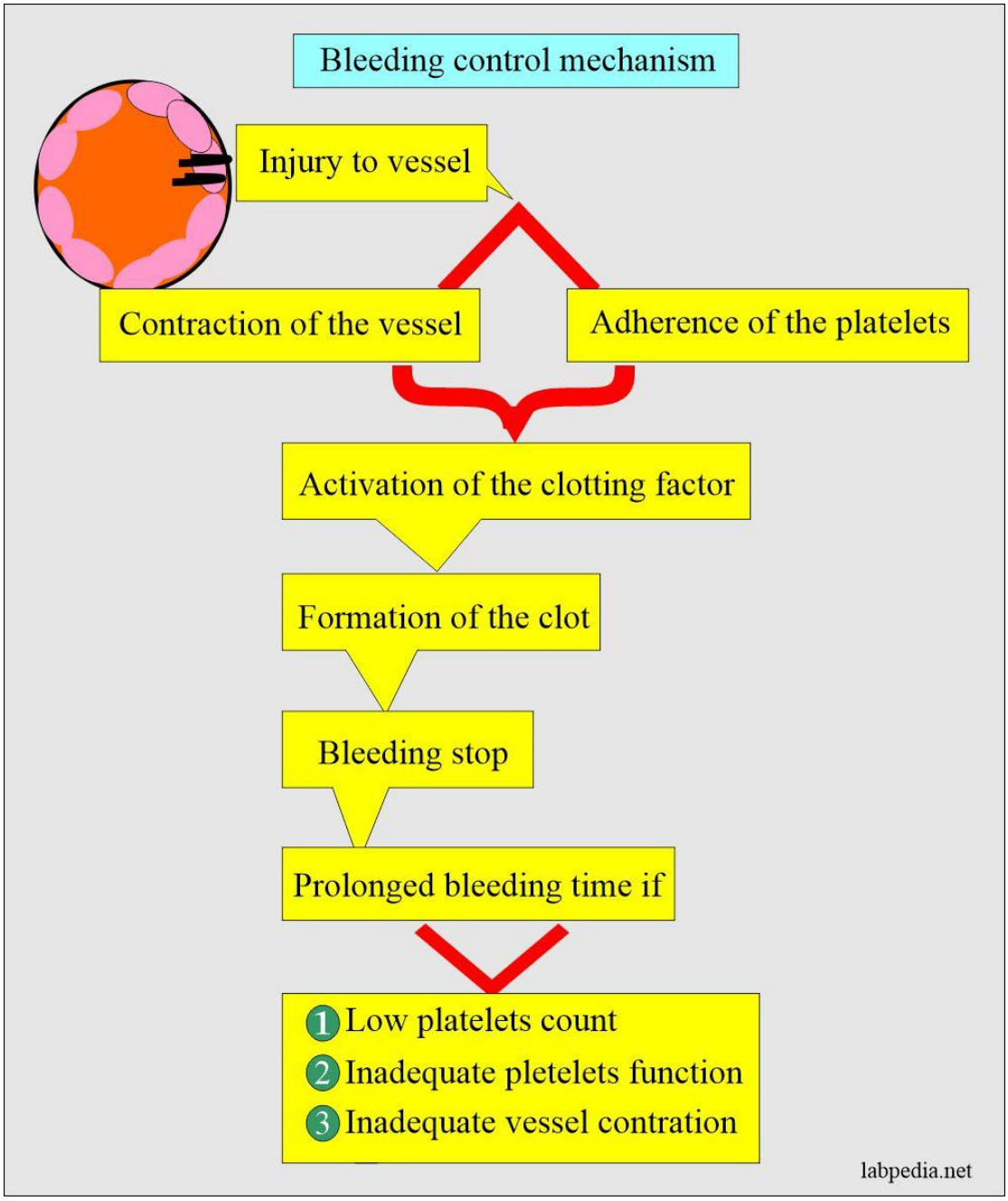
1. Prick the finger with the lancet.
2. Hold the capillary over the blood, and the capillary will fill automatically.
3. Now, after regular intervals, break the capillary.
4. When a clot starts forming, that is the endpoint and clotting time.




Blood clotting mechanism
Three stages
Vascular spasm
Platelets plug formation
Coagulation factors activation
Stages of blood clotting
Formation of prothrombinase:
1. Intrinsic pathway
2. Extrinsic pathway
Prothrombine
Thrombine (enzyme)
Fibrinogen (soluble)
Fibrin (insoluble)
labpedia.net

Bleeding Time (BT)

Bleeding time is a medical test done on someone to assess their platelet function , count and integrity of the blood vessels.



procedure

1-Warm up the finger for skin puncture.

2- Make an incision with a sterile disposable lancet to depth 3 mm.

3-As soon as blood is visible start the stop watch.

4-Wipe off the first drop of blood.

5-When blood stop from finger we take a time from stop watch.

Blood pressure

Arterial blood pressure is most commonly measured via a sphygmomanometer, which historically used the height of a column of mercury to reflect the circulating pressure. Blood pressure values are generally reported in millimetres of mercury (mmHg), though aneroid and electronic devices do not contain mercury.

For each heartbeat, blood pressure varies between systolic and diastolic pressures. Systolic pressure is peak pressure in the arteries, which occurs near the end of the cardiac cycle when the ventricles are contracting. Diastolic pressure is minimum pressure in the arteries, which occurs near the beginning of the cardiac cycle when the ventricles are filled with blood. An example of normal measured values for a resting, healthy adult human is 120 mmHg systolic and 80 mmHg diastolic (written as 120/80 mmHg, and spoken as "one-twenty over eighty").

Lung Volumes

Lung volumes are also known as respiratory volumes. It refers to the volume of gas in the lungs at a given time during the respiratory cycle. Lung capacities are derived from a summation of different lung volumes. The average total lung capacity of an adult human male is about 6 litres of air. Lung volumes measurement is an integral part of pulmonary function test. These volumes tend to vary, depending on the depth of respiration, ethnicity, gender, age, body composition[1] and in certain respiratory diseases. A number of the lung volumes can be measured by Spirometry- Tidal volume, Inspiratory reserve volume, and Expiratory reserve volume. However, measurement of Residual volume, Functional residual capacity, and Total lung capacity is through body plethysmography, nitrogen washout and helium dilution technique.

Lung Volumes :

- **Tidal Volume(TV)**

It is the amount of air that can be inhaled or exhaled during one respiratory cycle. This depicts the functions of the respiratory centres, respiratory muscles and the mechanics of the lung and chest wall.

The normal adult value is 10% of vital capacity (VC), approximately 300-500ml (6-8 ml/kg); but can increase up to 50% of VC on exercise .

- **Inspiratory Reserve Volume(IRV)**

It is the amount of air that can be forcibly inhaled after a normal tidal volume.IRV is usually kept in reserve, but is used during deep breathing. The normal adult value is 1900-3300ml.

- **Expiratory Reserve Volume(ERV)**

It is the volume of air that can be exhaled forcibly after exhalation of normal tidal volume. The normal adult value is 700-1200ml. ERV is reduced with obesity, ascites or after upper abdominal surgery

- **Residual Volume(RV)**

It is the volume of air remaining in the lungs after maximal exhalation. Normal adult value is averaged at 1200ml(20-25 ml/kg) .It is indirectly measured from summation of FRC and ERV and cannot be measured by spirometry.

n obstructive lung diseases with features of incomplete emptying of the lungs and air trapping, RV may be significantly high. The RV can also be expressed as a percentage of total lung capacity and values in excess of 140% significantly increase the risks of barotrauma, pneumothorax, infection and reduced venous return due to high intra thoracic pressures as noticed in patients with high RV who require surgery and mechanical ventilation thus needs high peri-operative inflation pressures.

Lung capacities

- **Inspiratory capacity(IC)**

It is the maximum volume of air that can be inhaled following a resting state. It is calculated from the sum of inspiratory reserve volume and tidal volume. IC = IRV+TV

- **Total Lung Capacity(TLC)**

It is the maximum volume of air the lungs can accommodate or sum of all volume compartments or volume of air in lungs after maximum inspiration. The normal value is about 6,000mL(4-6 L). TLC is calculated by summation of the four primary lung volumes (TV, IRV, ERV, RV).

TLC may be increased in patients with obstructive defects such as emphysema and decreased in patients with restrictive abnormalities including chest wall abnormalities and kyphoscoliosis.

- **Vital Capacity(VC)**

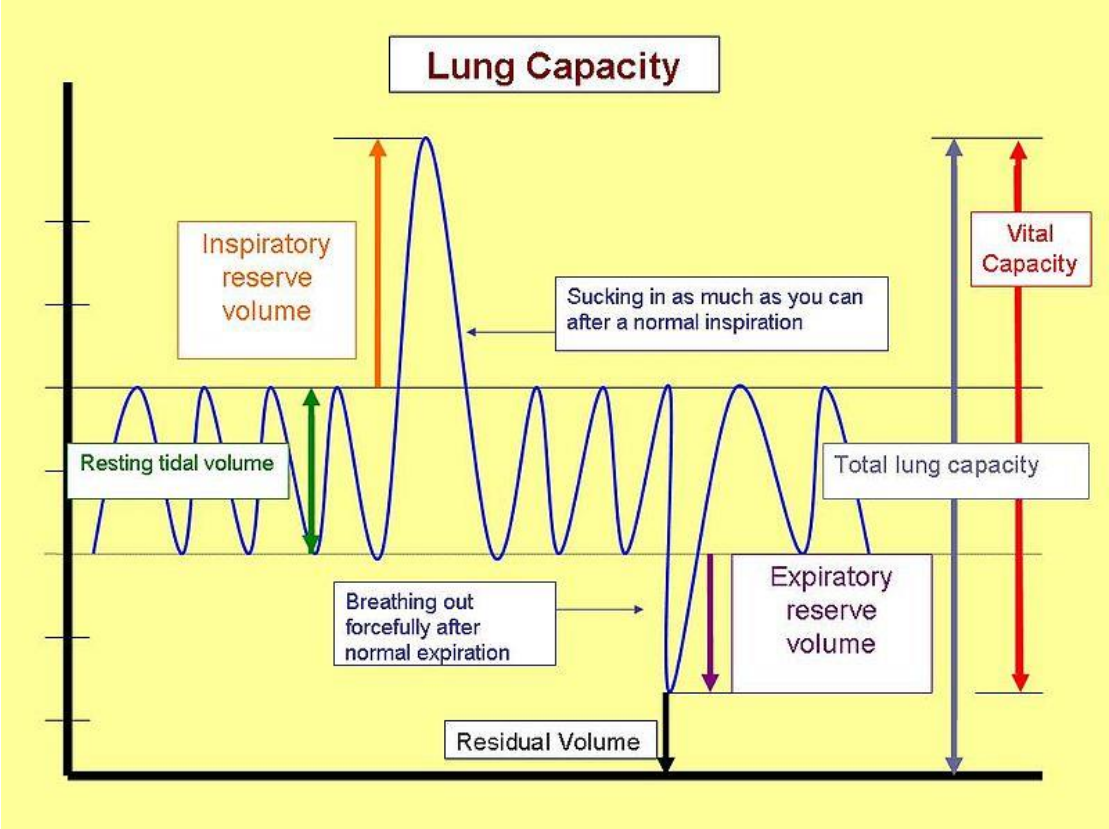
It is the total amount of air exhaled after maximal inhalation. The value is about 4800mL and it varies according to age and body size. It is calculated by summing tidal volume, inspiratory reserve volume, and expiratory reserve volume. VC = TV+IRV+ERV.

VC indicates ability to breathe deeply and cough, reflecting inspiratory and expiratory muscle strength.VC should be 3 times greater than TV for effective cough. VC is sometimes reduced in obstructive disorders and always in restrictive disorders

- **Function Residual Capacity(FRC)**

It is the amount of air remaining in the lungs at the end of a normal exhalation. It is calculated by adding together residual and expiratory reserve volumes. The normal value is about 1800 – 2200 mL. FRC = RV+ERV.

FRC does not rely on effort and highlights the resting position when inner and outer elastic recoils are balanced. FRC is reduced in restrictive disorders. The ratio of FRC to TLC is an index of hyperinflation. In COPD, FRC is upto 80% of TLC.



Links

https://www.youtube.com/watch?v=uSq0-0W5vOk

https://www.youtube.com/watch?v=0f9p9JX4qJk

https://www.youtube.com/watch?v=Pxmt8FdDqN4

https://www.youtube.com/watch?v=A2Jr-9ylzzA

https://www.youtube.com/watch?v=SoWXhpZC1NA

https://www.youtube.com/watch?v=amJAJeWk0NI

https://www.youtube.com/watch?v=b2dZn1HVT-k

https://www.youtube.com/watch?v=odQ6ggM67Ck