WBC's count ☺

**# Introduction**

**White blood cell count** : is the count of total number of leukocytes in a volume of blood, expressed as WBC's/1 mm3 blood .

# WBC's or leukocytes are :

1- colorless \*

2- nucleated cells

# They are formed from stem cells in the bone marrow

\*\* **Function** : they protect the body tissues by engulfing disease-bearing bacteria and foreign matter , by a process called **phagocytosis** .

**#principle of the text :**

# Because the number of WBC's in a cubic millimeter of blood is large , it is practical a sample of blood with **a diluting solution** “ 2% Glacial acetic acid with methylene blue “

\*\* We use a Glacial acetic acid to lysis all cytoplasmic membrane , therefore leaving the nuclei of WBC's .

 \*\*Nucli of WBC's are colorless so for watch them under microscope we use a dye called methylene blue

\*\* 2% >> the percentage of diluting

**#Experience tools**

1] blood sample

2] WBC's diluting pipette

3] hemocytometer

4] diluting fluid ( 2% acetic acid & methylene blue

5] microscope

**# WBC's diluting pipette :**

WBC's pipette is special pipette consist of stem and blub , it have three marks ( 0.5 ; 1.0 & 11 ) and usually have a white bead .



**#The hemacytometer :**

\*\* the hemacytometer counting chamber is used for cell counting .

\*\* the distance between the bottom of the cover slip and the surface of the counting area of the chamber is **0.1 mm ( 1/10 mm )**

\*\* the surface of the chamber contains **two square ruled areas** separated by an H-shaped moat , these two square are identical , allowing the technologist to duplicate the cell count .

\*\* **Each** has a total area of **9 mm2** these squares are dividing into **nine primary\* squares with an area of 1 mm2 each** .

\*\* **The four corner primary squares** are used when counting **leukocytes** , each of them contain **16 secondary ( medium ) squares.**

\*\* The **central primary squar** is used **for RBC's count** , it consist **of 25 secondary (medium)** squares , and **each** of these 25 squares is further **divided into 16 smaller tertiary (smallest ) squares** .

 **# specimen ( EDTA-anticoagulated blood or capillary blood is preferred .)**

**#procedure :**

1] with a safety bulb draw blood up to 0.5 marks on WBC's pipette snd complete to 11 with WBC's diluting solution

2] mix for 2-3 minute

3] charge hemacytometer

Load the counting chamber with diluting blood as follows :

\*\* discard the first 4-5 drop

\*\*place tip of pipette at edge of the central platform of hemacytometer slide and let a drop of diluted blood run between the hemacytometer slide and cover slip by capillarity

4] let the hemacytometer to stand on the bench for 3-5 minute so the celle are settled down

**#count & calculate :**

\*\* place the hemacytometer on the microscope stage and focus with the 4X objective , on the ruled area , move to the 10X and focus on one of the large corner square .

**\*\* counting :**

Count the cells in each large squares starting from ***the upper left medium square*** and follows the pattern indicated by the arrow in following square .



Count the cells within each square including cells touching the line at the top and on the left . cells touching the line on the right and at the bottom should not be counted \*\*.

Get the total number of cells counted in all four large squares .

**# calculation :**

**Number of cells/cubic mm blood = counted cell in 4 large square \* dilution factor \* volume correction factor**

Dilution factor = 20

Volume correction factor = 2.5

شوفوا ال calculation من المانيوال ص 10 :P

ال dilution w volume correction factor ثابتات بس احفظوا ال calculation احتياط اذا طلب شي عنهم :3

 **Normal values :**

**Newborn 9000-30000 cell/cu mm blood**

**Adult 4000-11000 cell/cu mm blood**

**\*\* Significance of the test :**

There are two important definition related to the leckocytes :

**1-leukocytosis:** a condition when total number of WBC's exceeds normal WBC's count ( 11000 cells/ mm3 ) and occur as an indicator of body defense against foreign materials ( bacteria , parasites & toxins ) .

**2- leucopenia :** a condition when the total number of WBC's below normal ( 4800 cells/mm3 ) and result from x-ray therapy , Alcoholism , Antibiotic therapy , typhoid infection , measles , infectious hepatitis , tuberculosis , and cirrhosis of the liver .

**#sources of error :**

1] flooding of chamber with excess sample

2] failing to count all the cells in the square or conversely including artifacts the count .

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