MANUAL RBC’s COUNT

INTRODUCTION :
-some information that we have already studied in physiology about the red blood cells ( Erythrocytes ) :

\*they are biconcave in shape .

\*have no nucleus .

\*they contain red pigments called **hemoglobin** which is responsible for the red color of the blood .

\*they live for 120 days .

\*\* a healthy *male* has about 4.5 – 6 million of RBCs per **cubic millimeter ( mm3 )**

And a healthy *female* about 4 – 5.5 million .

- IN THIS LAB :
we want to determine the number of RBCs in a cubic millimeter of blood

\*BUT because the number of RBCs is very large we will dilute( ( نخفف the sample of the blood .

\* for the dilution we will use isotonic solution like ( Hayem’s solution ) .

Note :
in the exam he can mention the dilute solution in three ways and they all give the same meaning ..

\* ISOTONIC SOLUTION –\* HAYEM’S SOLUTION –\* NORMAL SALINE

- HOW WE COUT :

a) THE MATERIALS we will need :

Fig. 1

- Blood sample.

- RBCs diluting pipette ( fig. 1 ) .

- diluting fluid ( as we mention we will use Hayem’s solution ) .

Fig. 2

- hemocytometer (fig. 2 ).

-and of course a microscope ☺

+ the RBCs diluting pipette :

\*it’s a special pipette consists of stem and bulb and usually have red bead .

\* the whole volume of the pipette is 101 **unit volume** .

Notice that WBCs diluting pipette is smaller than the RBCs one.. Its whole volume 11 unit volume
also another different is that the WBCs pipette have white bead ( look at the fig. 1 )

+ the hemacytometer :

\* the hemacytometer chamber is used for counting the cells ( as in the fig. bellow ) 

\* the surface of the chamber contains TWO IDENTICAL squares and they are separated by an H – shaped moat (زي الخندق )

\* because these two squares are identical they allow technologist to duplicate the cell count .

\* each square has a total area of 9 mm2 ( مساحته ) 3 x 3 .

\*these sqares are divided into nine primary squares with an an area of 1 mm2 .

Note:

we will just use the central primary square for RBCs count .
it consists of 25 secondary squares and each one of these 25 squares is divided into 16 smaller squares



- **PROCEDURES:**

1- put the blood in the RBCs pipette up to 0.5 mark .

Note:
- it’s not necessary to put 0.5 of blood we can put blood up to 1 unit volume.
**So** in the exam he may mention another number instead of 0.5 BUT if he doesn’t mention any number then assume that the number is 0.5.

- We use EDTA as an anticoagulant for blood ( مسيل للدم ) .

2- let the blood reach to pipette .

3- put the hayems solution in the pipette up to 101

4- mix for 2-3 min.

5- now we will fill the hemacytometer :
\*dicard the first 4-5 drops
\* place the tip of the pipette at the 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 ( الخاصية الشعرية ) ( fig. 3 )



 Fig. 3

6- let the hemacytomater to stand on the bench for 3 -5 min. SO the cells are settled down

Note :
-the distance between the cover slip and the surface of the counting area of the chamber is 0.1 mm . 

-JUST understand these procedures that I mentioned وما تحكولي صعب :P

- COUNT and CALCULATION:

A- \* Under the microscope first we use 40 x magnification to see the 9 squares and then determine the central square

 \* from that square we only need the the 4 corner square and the central one and to determine these 5 squares we will use 100 x magnification ( fig.4 )

 \* to see each one of these 5 squares alone we will use 400x magnification

 \* we count all cells that touch the upper and left line AND don’t count any cells that touches a lower or right line ( for **example** look at fig. 5 )

 \* نعد بطريقة " الزقزاق "



Fig. 5

Fig. 4

B- **CALCULATION:**

The method that I will mention is different from the book BUT its easier and its from the teacher so don’t worry

\*\* the low : number of RBCs = ΣR1 and R2 X D.C.F X V.C.F
 ( or any two R )

D.C.F ( dilution correction factor ) = =  = 200

V.C.F ( volume correction factor ) = 5 تعبر عن الخمس مربعات ) )
volume of R ( the volume of the small square ) = الطول x العرض x الارتفاع = 0.2 x 0.2 x 0.1 = 0.004 MM3

**Note**: the Height is for the coverslip ( ارتفاع الغطاء )

V.C.F = = 50

For example : #RBCs =ΣR1 and R2 X D.C.F X V.C.F

 = 500 x 200 x 50 = 500.000

\*\* I tried my best to make this clear if u don’t understand it u have to see the book ☺

\* IF the number of RBCs is lower than normal then we have **anemia

\*** if the number of RBCs is higher than normal then we have **polycethemia**

**\*\* polycethemia is 2 types :**

**1- primary polycethemia : RBCs over 14 million cells per mm3**

 - Occure in bone marrow malignancy

2- **secondary : polycethemia** : RBCs is 8 million cells per mm3

 - its caused by : \*respiratory disease

 \* heart disease

 \* chronic carbon dioxide poisoning

**بذلت ما في وسعي لتبسيط الكتاب وادراج كل ما ذكره الدكتور في شرحه .. سامحوني على تقصيري وارجو من كل قلبي ان تستفيدوا ^^**

DONE BY : HARETH INAYA ☺

EDITED BY : Hazem Mohamed