ALQUSOUR ACADEMY
اكاديمية القصور

Q.A.J.U.S.T

PHYSIOLOGY LAB

لطلبة العلوم الطبية والصيدلة

Subject:

First Exam: Lab Material

ALQUSOUR ACADEMY

خاص
للفصل الدراسي الأول
2015 - 2014

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ساعـــات الـدوام الرسمـي

المقر الرئيسي
السبت - الخميس: 11:00 ظهراً - 12:00 ليلاً
الجمعـــــة: 2:00 ظهراً - 12:00 ليلاً

مقر ش. الجامعة
السبت - الخميس: 12:30 ظهراً - 9:30 ليلاً
الجمعـــــة: عطلة

تحذير: محاضراتنا (الملخصات) متوفرة لدى:

1 - أكاديمية القصور بفروعها.
2 -جمعية التصوير الطبية (مدرج التمريض).

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WBC's & RBC's Count

- **WBC's or RBC's count**: It's the count of total number of leukocytes or erythrocytes in a volume of blood, (RBC/ 1 mm^3, WBC/1 mm^3) blood.

- **WBCs "leukocyte":**

1- Colorless, nucleated cells.
2- Formed from stem cells in bone marrow.
3- Main function: Defense mechanism "Phagocytosis".
4- Normal range: Newborn (9000-30000 cell/mm^3 blood).
Adult (4000-11000 cell/mm^3 blood).

- **RBCs "erythrocyte"**:

1- No nucleus.
2- Biconcave in appearance.
3- Contain pigment called hemoglobin.
4- Function: to combine with O_2 and to lesser extent with CO_2 and transport them through the blood vessels.
5- Life span: 120 days.
6- Normal range: Male (4.5-6 million/mm^3 blood).
Female (4-5.5 million/mm^3 blood).
Newborn (up to 8 million/mm^3 blood).

- **Material used in both procedures**:

1. **Blood sample** (EDTA-Anticoagulated blood or capillary blood is preferred)
2. **WBC's diluting pipette**, has three marks (0.5, 1.0 and 11), and has a white bead.
   RBC's diluting pipette, has three marks (0.5, 1.0 and 101), and has red bead.
3. **Diluting fluid**: For **WBCs** count is **2% of acetic acid with methylene blue.**
   For **RBCs** count is **Hayem's solution.**
4. **Microscope.**
5. **Hemocytometer:** A modified glass-slide that has a counting chamber, used for cell counting. We use **Neubauer** hemocytometer.
   - It's constructed so that the distance between the bottom of the cover slip and the surface of the counting area is **0.1mm (1/10 mm)**.
   - Surface of the chamber contains 2 squared ruled areas (identical) separated by H- shaped moat.
   - **Each square has total area 9mm^2** --- divided into **nine primary squares**, with an area of **1mm^2** of each.
   - **Four large corner squares** ---- **each one contains 16 secondary squares**, and these four squares are used for **WBCs counting**.
   - **Central primary square**--- used for RBCs counting--- consists of **25 secondary squares, each are divided into 16 tertiary squares**.

- **Procedure**: for both WBCs & RBCs count, same steps with some differences

1- With a safety bulb draw blood up to 0.5 marks on **WBC's** pipette. Complete to **11** with WBC's diluting solution, but in **RBC's** draw blood up to 0.5 mark on RBC's pipette and complete to **101** with RBC's diluting solution.

2- Mix for 2-3 minutes, for both.
3- Charge Hemocytometer.

- Load the counting chamber with diluted blood as follows: (in both)
  1- Discard the first 4-5 drops.
  2- Place tip of the pipette at edge of the central platform of hemocytometer slide and let a drop of diluted blood run between the hemocytometer slide and cover slip by capillarity.
  3- Let the hemocytometer stand on the bench for 3-5 minutes so the cells are settled down.

- **Count and Calculate**:
  1- In counting **WBCs** --- use magnification = **10X**, BUT in counting **RBCs**--- use **40X**.
  2- Counting: In WBCs-- - Count the number of cells in the four large corner squares.
  - Count the cells in each large square starting from the upper left medium square.
  - Count all the cells within each square, including cells touching the line at the top and on the left, BUT, cells touching the line on the right and at the bottom should not be counted.

- Also in RBCs counting, the same steps and rule, But, you count the cells in the 5 medium squares at the corners and the center of the large square in the center, each of these contains 16 (tertiary) squares---- total 80 squares.

3- Calculation: (in both procedures)

1- Number of cells/cubic mm blood =
Counted cells in all squares needed x dilution factor x volume correction factor

2- **The dilution factor (WBCs) = total volume/ sample volume**
= 11-1/0.5
= 20 (dilution factor of WBCs)

The dilution factor (RBCs) = 101-1/0.5
= 200 (dilution factor of RBCs)

3- **Volume correction factor = Desired volume/ counted volume**
= $1mm^3$ / counted volume

Total volume of the four large squares (WBCs) =Volume x Number of large squares
= (Width x length x depth) x 4
= (1mm x 1mm x 1/10mm) x 4
= 0.4 mm^3

Volume correction factor (WBCs) = 1/0.4 = 2.5

Total volume of the five medium squares (RBCs) = Volume x number of squares
= (Width x length x depth) x 80
= 1/20mm x 1/20mm x 1/10mm x80
= 1/50 mm^3

Volume correction factor (RBCs) = 1/ (1/50) = 50

Examples:

1. If total number of WBCs counted in the four large corner squares is 120. What is the WBCs count in this patient? **(Answer: 6000 cells/mm^3).**
2. If total number of RBCs counted in the five medium squares is 423. What is the RBCs count in this patient? **(Answer: 4,230,000 cells/mm^3).**

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- **Sources of errors:** (In both WBCs & RBCs)
1- Flooding of chamber with excess sample
2- Failing to count all the cells in the squares or conversely including artifacts in the count.

- Significance of the procedure:

1- There are two important definitions related to the leukocytes:

- **Leukocytosis ---- WBCs count > (11000 cell/mm^3)**

Causes:

1- **Body defense against "bacteria, parasite, toxins.**
2- **Metabolic disorder.**
3- **Chemical and drug poisoning.**
4- **Acute hemorrhage.**

- **Leukocytopenia ---WBCs count < (4800 cell/mm^3)**

Causes:

1- **X- ray therapy.**
2- **Alcoholism.**
3- **Antibiotic therapy.**
4- **Typhoid infection.**
5- **Measles.**
6- **Infectious hepatitis.**
7- **Tuberculosis.**
8- **Cirrhosis of liver.**

2- Disorder related to RBCs:

- **Anemia** ---- Decreased RBCs under normal level.
- **Polycethemia** ------ Increase in RBCs above normal level.

Two types:

1- **Physiological Polycethemia, up to 8 million/mm^3**

Causes:

- **Age** --- at birth RBCs count 8-10 million/mm^3
- **High altitudes**

2- **Pathological Polycethemia**: due to

- **Primary Polycethemia** – RBCs **>14 million/mm^3**, occurs in **bone marrow malignancy**.

- **Secondary Polycethemia** ---RBCs is **8million/mm^3**

Due to:

1- **Respiratory disease**
2- **Heart disease**
3- **Chronic CO_2 poisoning (smokers).**

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