EXPERIMENT II

Determination of hemoglobin concentration in blood
RBC, WBC & platelet counts

Determination of MCV, MCH, MCHC

Determination of HCT/PCV

Hemolysis & fragility test of RBC

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Purpose

- Learn how to determine the hemoglobin concentration in blood.
- Learn how to perform RBC, WBC & platelet counts.
- Learn how to determine MCV, MCH & MCHC.
- Learn how to measure PCV.
- Investigate the effects of NaCl solutions of various osmotic pressures on the morphology of RBCs & hemolysis.
Determination of hemoglobin concentration of blood

- In clinical practice, hematological information accumulated from a series of blood tests conducted on a small volume of blood - even a single drop - can be of great diagnostic and prognostic value.
- The hemoglobin content of a solution can be estimated by measuring its color, its power of combining with oxygen or carbon monoxide, or by its iron content.
- The most popular methods for hemoglobin estimation in modern clinical laboratories are colorimetric.
Red Blood Cell Count

- Total RBC & WBC counts are a routine part of any physical exam, & most clinical agencies use computers to conduct these counts.

- Hemocytometer. There are three kinds of hemocytometer: Burker, Thoma & Neubauer. The improved Neubauer counting chamber is the most convenient & is now widely used clinically.
Neubauer Counting Chambers
Count the RBCs in 5 medium squares (4 at the 4 corners & 1 in the center) inside the central large square. Each border of a medium square is marked with three close parallel lines (inner, middle & outer lines). The RBC count is made only within the square bordered by the middle line.
Count those cells which touch the upper & left medium lines of the medium squares but not those which touch the lower & right lines.
EXAMPLE

Total RBC count in 5 squares = 600
Multiply $600 \times 5 = 3000$ (Total number of cells in central square)
Multiply $3000 \times 10 = 30,000$ (Cells per mm$^3$)
Multiply $30,000 \times 200 = 6,000,000$ (Dilution factor of 200)
Total number of RBC in sample is 6,000,000 cells/ml
White Blood Cell Count

- **LEUKOCYTOSIS**
  An abnormally high WBC count, above 11,000 cells/mm³, may indicate bacterial or viral infection, metabolic disease, hemorrhage, or poisoning by drugs or chemicals.

- **LEUKOPENIA**
  A decrease in the white cell number below 4000 cells/mm³ may indicate typhoid fever, measles, infectious hepatitis or cirrhosis, tuberculosis, or excessive antibiotic or X-ray therapy. A person with leukopenia lacks the usual protective mechanisms we would expect to find in the immune system.
Platelets are cell fragments of large multinucleate cells (megakaryocytes) formed in the bone marrow. They appear as darkly staining, irregularly shaped bodies interspersed among the blood cells.

The normal platelet count in blood ranges from 250,000 to 500,000 per cubic millimeter.

Platelets are instrumental in the clotting process that occurs in plasma when blood vessels are ruptured.
Hematocrit (HCT)/packed corpuscular volume (PCV)

- When heparinized blood (heparin is an anticoagulant) is centrifuged, the red blood cells become packed at the bottom of the tube, while the plasma is left at the top as a clear liquid. The ratio of the volume of packed red cells to the total blood volume is called the hematocrit.
Determination of MCV, MCH, MCHC

MCV can be calculated as HCT/RBC count.

MCH is a calculated value and is defined as HGB/RBC giving the mean HGB concentration in the RBC population.

MCHC is a calculated value and is defined as HGB/HCT.
Hemolysis & fragility test of RBC

- The permeability properties of a membrane can be studied by testing whether various compounds or agents, chemical or physical, can permeate the membrane. One method of doing this is to observe the osmotic effects of compounds on the cell membrane.
- RBCs require a rather stable osmotic environment, but normal RBCs resist hemolysis due to the fact that the cells are bi-concave.
Hypertonic environment
Outflux of water

• CRENATION
• On the other hand, in a hypotonic environment (e.g. 0.4% NaCl or distilled water), an influx of water occurs: the cells swell, the integrity of their membranes is disrupted, allowing the escape of their hemoglobin (hemolysis) which dissolves in the external medium.

HEMOLYSIS
Experimental Procedure

• Rabbit operation
  (1) Catch
(2) Weigh

(3) Anaesthetize: 20% Urethane (ip) with the proportion of 5 ml/kg.

(4) Fix
Operation

Cut off the hair on the neck. Cut and separate the skin and the hypodermis in the front of neck about 8~10 cm in length. Separate the muscles with the hemostasia clamps. Separate its trachea and the connective tissues beside the trachea to find the left common carotid artery.
• Catch
• Weigh
• Anaesthetize
• Fix
• Operate
  • Separate the left common carotid artery.
  • Ligate the common carotid artery near the head end.
    keep a ligature thread for future use.
  • In order to stop the blood flow, clamp the common carotid artery near the cardiac end with a clamp.
  • Cut a small incision near the ligature thread.
  • Insert a canula into the common carotid artery from the incision toward the heart.
  • Ligate and fix the canula of the common carotid artery.
  • Inject 1000U/kg of **heparin** into the vein of outer ear.
Part I

Hemolysis & fragility test of RBCs

1. The test tubes are ranked in the test tube rack & are identified by numbers. Add 1% NaCl solution & distilled water accurately into each tube according to Table 2-1 & then shake evenly & gently.

Table 2-1 Hypotonic solutions

<table>
<thead>
<tr>
<th>Tube number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 % NaCl (ml)</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>DW (ml)</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>NaCl (%)</td>
<td>0.70</td>
<td>0.65</td>
<td>0.60</td>
<td>0.55</td>
<td>0.50</td>
<td>0.45</td>
<td>0.40</td>
<td>0.35</td>
<td>0.30</td>
<td>0.25</td>
</tr>
</tbody>
</table>
2. Take the fresh blood, add 1 drop (50ul) into each tube, shake evenly & gently, & stand for 30 min at room temperature.

3. Judge the results:
   (1) The liquid of the upper level has no color & the bottom layer is muddy red, indicating no hemolysis.
   (2) The upper layer is salmon pink & the bottom layer is muddy red, indicating incomplete hemolysis. In this condition, the sodium chloride concentration reflects the minimum resistance of the RBCs.
   (3) The liquid is transparent red & the bottom has no deposited cells, indicating complete hemolysis. In this condition, the sodium chloride concentration reflects the maximum resistance of the RBCs.
Part II

- Determination of hemoglobin concentration in blood
- RBC, WBC & platelet counts
- Determination of MCV, MCH, MCHC
- Determination of HCT/PCV

- Remove the clamp and get the fresh blood sample about 0.3ml in the tube
- Measure all above items with a CA620 Hematology Analyzer (Medonic Cell Analyzer CA620/530, Sweden)
- Detection is accomplished using the electronic impedance principle and occurs in the orifice of the transducer. (www.boule.se)
Analyzing the Sample (open tube)

- Choose the operational mode with ‘Menu-Operate’ so that the last run sample is displayed.
- Aspirate the sample through the aspirating pipette by pressing the start lever behind the aspiration needle, see picture below.
- Remove the sample when the text ’Aspirating…’ disappears.
- The display shows the following sequences:
  Last Sample (= blank) Aspirating sequence (ca. 3 sec.)
The CA620/530 VET switches now to the following menu.

Sample displayed after ca. 53 seconds from aspiration:

Print the result.
Part III

- Inject 0.9% sodium chloride weigh×1% (ml) into the vein in the ear to increase the blood volume.
- Take the fresh blood sample within 1 minute after injecting sodium chloride.
- Analyzing the Sample and compare the change of hemoglobin concentration, RBC, WBC, platelet counts, MCV, MCH, MCHC and HCT/PCV.
Requirement for Experimental Report

Results
1. Stick the original data (in the report of the group leader)
2. Design and complete a table for the result of two analysis of hemoglobin concentration, RBC, WBC, platelet counts, MCV, MCH, MCHC and HCT
Discussion
When does the hemoglobin concentration of blood increase or decrease?
What is the function of RBCs? When does RBC count increase or decrease?
What are the differences between blood plasma & serum?
Which factors affect the osmotic fragility of RBCs?