Lab Protocols

Hemoglobinometry, Hematocrit, S.E.
Blood Cells Counting

v. 0.2

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Labs aim: Explore biology in context through brain and hands

LAB: Hemoglobinometry, Hematocrit, Sedimentation rate

AIM of the lab

- See and understand basic blood test
- See the different aspects of red blood cells evaluation
- Basics of oxygen transportation

REQUIRED KNOWLEDGE

- Blood, plasma, blood composition, RBCs, hemoglobin, methemoglobin, oxygenation and oxidation of hemoglobin, spectrophotometry, sample, blank, suspension stability

Hemoglobinometry

WHAT

Estimation of hemoglobin concentration in blood.
Physiologically cca: women 150 g/L, men 160 g/L (+/- 10 g/L)
→ Tells us about O_2 transporting capacity of blood

TASKS

- In three blood samples that are provided perform hemoglobin concentration measurement (at least three tests per each sample per class)
- Calculate MCHC once other necessary results are available. Which?

WHY …

… do we measure it?

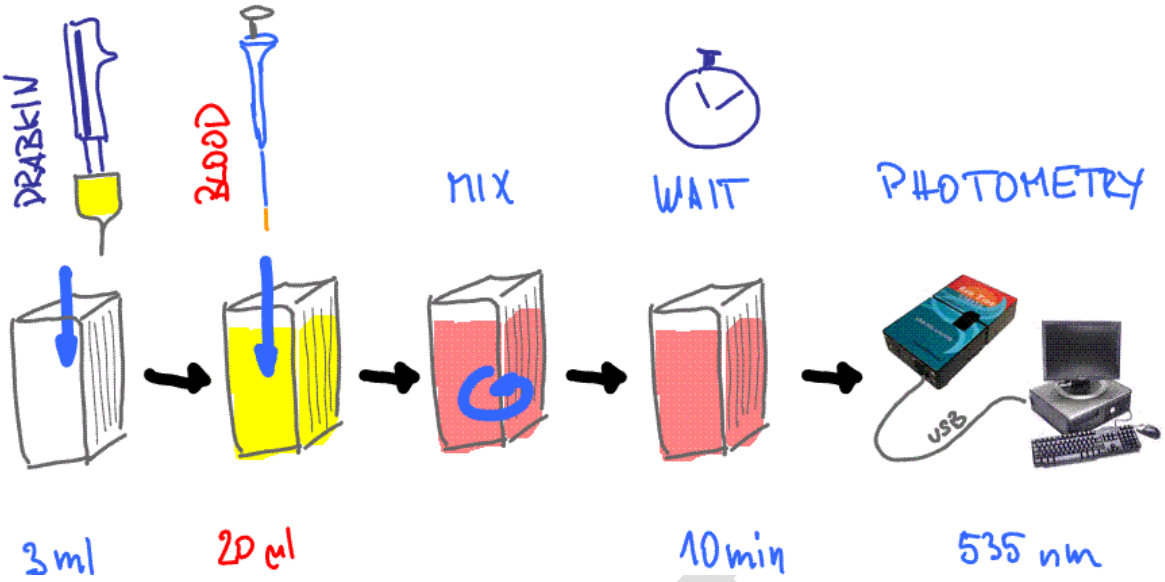
i. Amount of hemoglobin belongs among elementary tests of body's homeostasis. It crucially determines O_2 transporting capacity and thus O_2 delivery to the tissues.
ii. Pathologically, amount of hemoglobin can change, typically in anemias. Hb content can change independently of erythrocyte count and size (reflected by hematocrit). Knowledge of both hemoglobin content, number of RBCs and their size (volume) can help identify ethiology (reason) of anemia and thus suggest suitable treatment
iii. Critically low hemoglobin concentration will indicate, that transfusion might be necessary. More on transfusion e.g.: www.guideline.gov: Indications for and techniques of red cell transfusion

HOW

1. Colorimetry (visual assessment)
    - In principle, amount of Hb is proportional to colour of blood
    - Thus it is (theoretically) possible just to look and see ☺. Colorimetry is almost that simple ☺
    - Practically this method would be inaccurate, mainly due to:
        - Colur assessment Why?
        - Oxygenation of hemoglobin. Why?
    - Thus the procedure contains
        - Hb oxidation by HCl
        - Blood dilution
        - Comparison to standard colour (available in the set)
2. Photometry
    a. The principle remains the same as for colorimetry but (spectro)photometer is used for evaluation. Thus subjective assessment is excluded

Procedure:

i. Fill 10 cuvettes with Drabkin solution (**3 ml**). Why ten?
    1. contains K-cyanate (Why?)
    2. it is toxic (Why?)
ii. Take blood from samples **20 µl** and transfer to cuvettes
    1. How would you obtain such amount of blood?
iii. mix properly!!! (use pipette or glass stick)
iv. incubate 10 mins
v. mix again if sedimentation occurs (why?)
vi. perform photometry using Vernier photometer
    1. 565 nm
    2. use drabkin sol for blank
vii. calculate hemoglobin concentration
viii. what info is needed



3. gasometry (not performed)
    a. Principle:
        i. blood is fully saturated by O_2 (how?)
        ii. blood is fully desaturated
        iii. amount of released O_2 during desaturation is measured

RESULTS:

	extinction				HB conc
Sample	**test 1**	**test 2**	**test 3**	**avg**	**[g/l]**
1					
2					
3					

QUESTIONS:

1. What could be the sources major errors during the procedure?
2. Which of the methods would NOT be suitable in methemoglobinamemia?

Hematocrit (HCT)

WHAT

1. *medline:*
   The (relative) volume of packed RED BLOOD CELLS in a blood specimen. The volume is measured by centrifugation in a tube with graduated markings, or with automated blood cell counters. It is an indicator of erythrocyte status in disease. For example, ANEMIA shows a low value; POLYCYTHEMIA, a high value.

2. *easy words:*
   Percentage of volume of erythrocytes out of total volume of blood.
   Physiologically cca 45% (+/- 5 %)
   → Tells us about blood fluidity (viscosity) and more

$$HCT = \frac{\text{Vol of RBCs}}{\text{Vol of Blood}}$$

Use *A B C* from the picture to fill the equation: *HCT* = ———

TASKS

In three blood samples that are provided estimate hematocrit
(Perform at least three tests per each sample - per class)

WHY …

- … do we check hematocrit? this all?
  i. Generally, these belong among elementary tests of body's homeostasis. Most in-door patients would have it done on admission.
  ii. Specifically HCT, Hb and RBCs count allow us to distinguish among different anemias.
  iii. Proportion between RBCs and plasma determines blood fluidity. Lower hematocrit means better fluidity and thus sometimes better tissue oxygen delivery.

HOW?

1. **Microhematrocrit (just from "a drop "of blood)**
   - Due to higher specific gravity of cells these tend to separate from plasma.
   - Spontaneous separation would be very slow if possible.
   - Thus centrifugation is used to speed the separation
   - Also, blood must be anticoagulated (Why?)
   - The anticoagulation must not interfere with the measurement.
     - How could it interfere?
       - …
       - …
       - …
     - Which anticoagulant would be suitable?

   Procedure:
   i. Find the samples 1, 2 and 3 and shake properly (why?)
   ii. For each sample fill at least 3 capillaries with blood
      1. Fill about 2/3 of capillary (who not much more / less?)
      2. How?

iii. Seal one end of capillaries in flame. Which end?
iv. Sealed capillaries place into the stand. Notice the positions!
v. Ask lab assistant to perform centrifugation.
NOTE: the capillaries will be returned in the same places as when handed to the assistant.
vi. Estimate hematocrit either using a ruler or the device in the lab.

RESULTS

	Hematocrit [%]				HB	MCHC
Sample	**test 1**	**test 2**	**test 3**	**avg**	**[g/L]**	**[%]**
1						
2						
3						

Calculated values for RBCs

- RBCs parameters such as count, Hb and HCT are somehow independent (RBC can be big but have low amount of Hb)
- In order to see whether the parametersch change proportionally or not some calculated values are used. They allow for "one-look" assessment of two params:

  - MCV = mean corpuscular volume = avg. volume of one erythrocyte

    $$MCV = \frac{HCT}{RBCcount},$$ norm cca 100 fl/ery

  - MCH = mean corpuscular hemoglobin = avg. Hb content in one ery.

    $$MCH = \frac{Hb}{RBCcount},$$ norm cca 30 pg/ery

  - MCHC = mean corpuscular hemoglobin concentration = avg. Hb concentration in erythrocytes

    $$MCHC = \frac{Hb}{HCT},$$ norm cca 35%

Blood sedimentation

(or Ery. sedimentation rate (ESR), or FW - Farheus Westergreen method)

WHAT

1. Medline: Measurement of rate of settling of erythrocytes in anticoagulated blood.
2. Medline Plus: ESR (erythrocyte sedimentation rate) is a nonspecific screening test for various diseases. This 1-hour test measures the distance (in millimeters) that red blood cells settle in unclotted blood toward the bottom of a specially marked test tube.

Physiologically cca 3-12 mm/h

WHY?

… do erythrocytes sediment?

… the test is performed?

- The erythrocyte sedimentation rate (ESR) can be used to monitor (progress of) inflammatory or malignant disease. Although it is a screening, *nonspecific* test (cannot be used to diagnose a specific disorder), it is useful in detecting and monitoring tuberculosis, tissue necrosis, rheumatologic disorders, or an otherwise unsuspected disease in which symptoms are vague or physical findings are minimal.

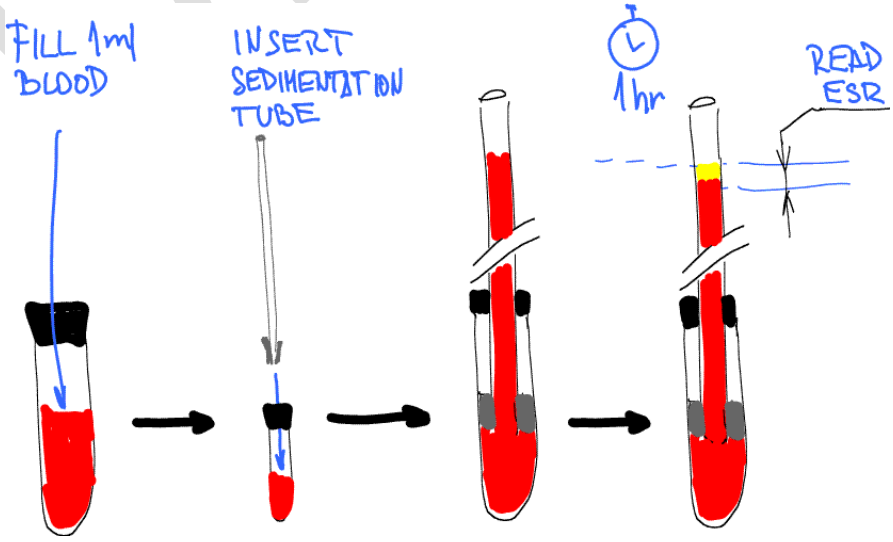
HOW?

… is ESR influenced?

- By many factors,
  - Elevated fibrinogen: helps formation of stacked erythrocytes called *rolleaux* that are relatively heavier and settle fastest.
  - Erythrocytes: number (anemia/polycytemia) size (macrocytosis/microcytosis, spherocytosis)
  -

… is Sedimentation measured?

- Blood withdrawn (How much? How?)
- Diluted and anticoagulated (anticoagulant prefilled in test-tube/syringe)
  - Which anticoagulant will be suitable?
- The sedimentation tube filled
- After one hour evaluate



LAB: Blood Cells Counting

WHAT

1. *Medline (MeSH ID D00177)*
   Blood cells count: The number of LEUKOCYTES and ERYTHROCYTES per unit volume in a sample of venous BLOOD.
   A complete blood count (CBC): also includes measurement of the HEMOGLOBIN; HEMATOCRIT; and ERYTHROCYTE INDICES.

2. *easy words:*
   Number of cells per volume of blood (not plasma! ;-)

REQUIRED KNOWLEDGE:

Composition of blood, Erythrocytes (number, volume, composition, function), Leucocytes (types, differential count, composition, funcrion), Plasma, Osmolarity, more;-)

PRACTICAL TASKS

In three blood samples that are provided cont RBCs and WBCs
(each student counts both RBCs and WBCs)

WHY …

- … do we count blood cells?
  i. Generally, these also belong among elementary tests of body's homeostasis. Many in-door patients would have it done on admission.
  ii. Helps to diagnose many diseases, monitor their progress
- … do we count RBCs?
  i. Specifically RBCs count (with HCT, Hb) allows us to discover and distinguish different anemias.
  Anemias are quite common, often unidentified, may accompany chronical diseases and may significantly affect prognosis
  ii. To help identify other hematological diseases (e.g. polycytaemia)
- … do we count WBCs
  i. WBC count (and differential count) is non-specific or semi-specific marker of inflammatory processes such as
     1. Infection (bacterial, viral, parazital)
     2. Allergy
     3. Autoimmunity
     4. Shock
     5. neoplasms
  ii. WBC count (and differential count) may be a specific sign of hematological neoplasms.

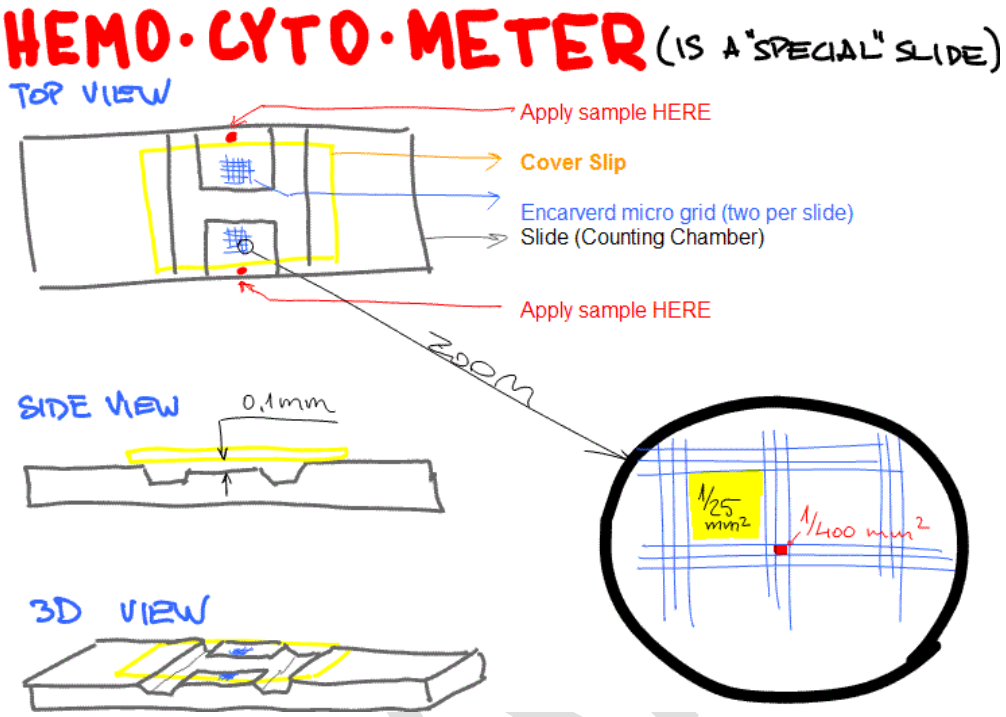
HOW?

1. **Counting chamber (e.g. Hemocytometer , Buerker chamber) and microscope**
   - Just a drop of blood is sufficient (Why only drop?)
   - Blood is diluted (Why?), anticoagulated (if necessary)
   - Blood cells stained and fixed if necessary (which cells need staining?)
   - ***Cells are counted visually under the microscope***
   - The volume in which the cells are counted needs to be known. Thus, specific slide called ***counting chamber*** or ***hemocytometer*** is used. (see the picture) Tiny grid is engraved into the slide and is seen under microscope only. Grid

determines *area*, space between slide and cover slip determines *height*. *Area* times *height* gives ***volume***.

- final blood count needs to be calculated (from # of cells, dilution and volue)

$$count = \frac{number\_of\_cells}{volume} \times dilution$$



2. **Automated machine (Flow cytometer) [NOT AVAILABLE IN THE LAB]**
   a. Blood sample is processed in machine (diluted, stained, etc.)
   b. Sample is forced through a tiny capillary where cells travel one – by – one. (What is the diameter of such capillary?)
   c. Each cell passing thru the capillary is detected and counted. (optically or electrically).
   d. Thus cells count can be calculated from: known volume of sample, dilution of blood,and passed cells.

Notes:

TASK

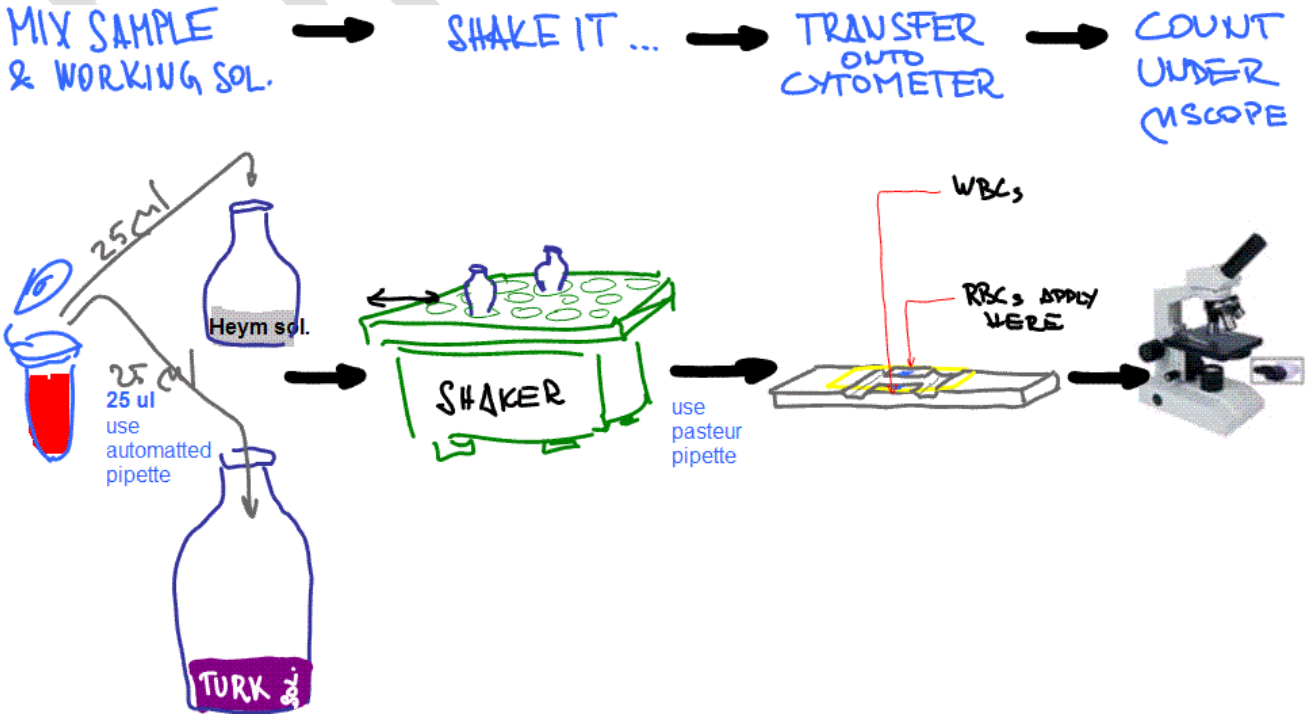
Estimatate blood cels count (both RBCs and WBCs) in given samples (Hemocytometer)
Each working group (students at one microscope) is supposed to process one sample.
Record and DISCUSS the results.

Procedure smmary:

Cells	Solution	dilution	Area for counting	Count in
RBCs	Heym's sol. - cells fixation - hypertonic - no staining	200x	Small squares 1/400 mm^2	80 squares
WBCs	Turck's sol. - stains WBCs nuclei - hemolyzes all cells (Why?)	20x	Large squares 1/25 mm^2	50 squares

.STEP-BY-STEP Procedure (Hemocytometer):

i. Find the blood samples 1, 2 and 3 and shake properly (why?)
ii. Transfer 25 µl of blood into pre-filled vile with respective working solution. (Heym solu. for RBCs, Turck solu. for WBCs).
Use automatic pipette to transfer blood/.
iii. Shake in the shaker for 10 minutes. (why?)
iv. Apply one drop of processed sample at the edge of cover slip as shown in the picture. One edge for RBCs, opposite one for WBCs.
v. Allow for sedimentation for 5 minutes (why???)
vi. Count cells under the microscope
    1. Recommended magnification 1:100
    2. Count RBCs above 80 smallest squares
    3. Cont WBCs above 50 large squares
ii. From data obtained calculate numbers of cells in original blood samples. The calculation must include:
    1. Number of cells obtained
    2. Volume in which counting occurred
    3. Dilution of the sample



RESULTS

Team (microscope)	Blood sample ID	RBC count [x10^6/µl]	WBC count [x10^3/µl]	interpretation
1				
2				
3				
4				
5				
6				
7				
8				

SUMMARY: