MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE

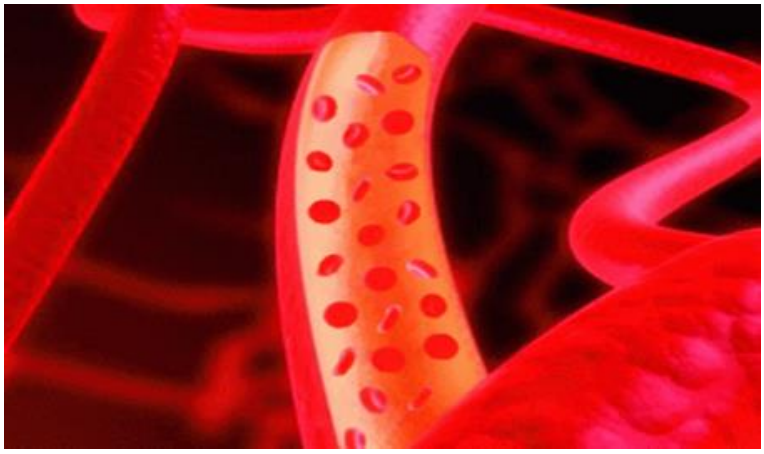
STEPAN GZHYTSKYI NATIONAL UNIVERSITY OF VETERINARY MEDICINE AND BIOTECHNOLOGIES LVIV

Faculty of Veterinary Medicine

Department of Normal and Pathological Physiology named after S. V. Stoianovskiy

PHYSIOLOGY OF THE BLOOD SYSTEM

Methodological instructions for laboratory classes of subject «Animal physiology» for foreign students of the speciality 221 «Veterinary medicine», branch of knowledge 21 «Veterinary Medicine»



Lviv 2022

UDK: 636.082.575(075)

Editor:
Iryna Kolomiiets PhD, Associated Professor, Department of Normal and Pathological Physiology named after S.V. Stoianovskiy, Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv.

Reviewer:
Andriy Tybinka Doctor of Veterinary Sciences, Professor, Professor of Department of Normal and Pathological Morphology and Forensic Veterinary, Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv

Kolomiiets Iryna. Physiology of the blood system. Methodological instructions for laboratory classes of subject «Animal physiology» for foreign students of the speciality 221 «Veterinary medicine», branch of knowledge 21 «Veterinary Medicine». – Lviv : Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, 2022.– 26 p.

In the methodological instructions are represented the main experiments of the chapter «Physiology of the blood system», handout for foreign students, which correspond to the syllabus of the educational discipline «Animal Physiology» for foreign students of the speciality 221 «Veterinary medicine», branch of knowledge 21 «Veterinary Medicine». They contain also list of questions for current and summary (examination) knowledge control. They include a list of methodological and recommended literature. The methodological instructions will be also useful for foreign students of medical and biological high schools.

The methodological instructions have been read and approved at the meeting of department of Normal and Pathological Physiology named after S.V. Stoianovskiy protocol №9 from the «15th» of February 2022, and approved by the decision of the educational-methodical commission of the Faculty of Veterinary Medicine, Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv protocol № 6 from the «17th» of February 2022.

CONTENTS

LABORATORY CLASS 1
TECHNIQUE OF BLOOD COLLECTION IN LABORATORY ANIMALS

Overview. Higher forms of animals have developed circulating blood, and the fluids derived from it, as a means of maintaining a relatively constant environment for all cells. Blood system consists of four parts: blood proper, hemopoietic organs, organs of blood destruction, and regulatory neurohumoral mechanisms. Blood, lymph and Interstitial fluid compose the internal medium of the organism.

Objectives. To become familiar with the procedure of technique of blood collection in laboratory animals in order to complete subsequent lessons with the following objectives.

Equipment. Laboratory animal (rabbit, guinea pig, rat, mouse), blood needles, cotton wool, 96 ° alcohol, anticoagulant (5% sodium citrate, heparin), glass test tubes.

Procedure. The following includes detailed instructions for the procedure of technique of blood collection in laboratory animals listed below. First of all the animals are securely fixed. The fixation is partial or complete immobilization of any animal. Then the hair is cut or shaved, the skin is wiped with an alcoholic solution of ether. A sterile needle is used to do prick or injection the skin, the vessel wall, and collect the appropriate amount of blood in a sterile test tube. If we need the whole blood we use an anticoagulant. First of all anticoagulant is poured into a test tube. After it blood is collected.

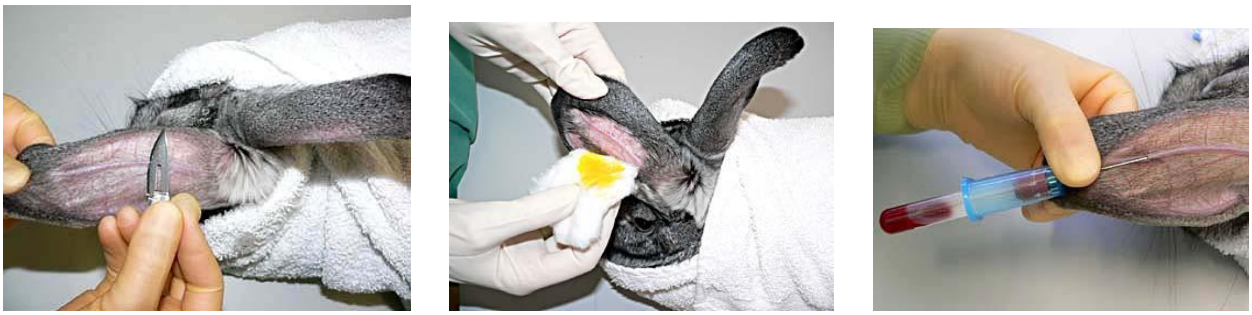


Figure 1. Technique of blood collection in rabbit

Примітка. Тут і далі джерело рисунків: Новосад Н.В. Лабораторні тварини і техніка біологічного експерименту: Навчально-методичний посібник для студентів біологічного факультету денного та заочного відділень (напрям підготовки: «Біологія»; галузь знань: «Природничі науки»). – Запоріжжя: ЗНУ, 2011. – 85 с.

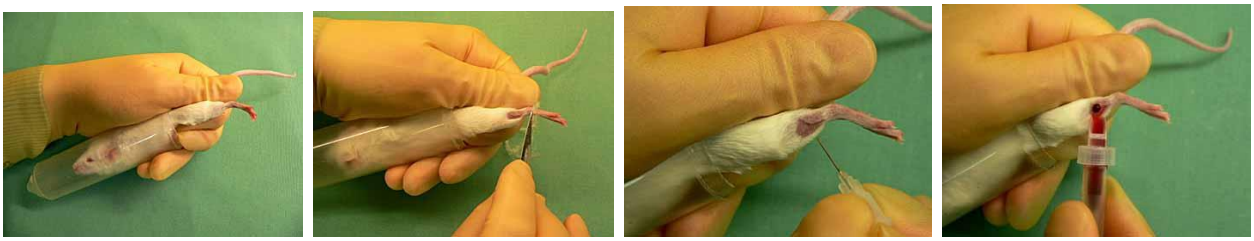


Figure 2. Technique of blood collection in mouse

Research results. In results students describe the differences in procedure of blood collection in laboratory animals.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 2
THE COMPOSITION OF BLOOD. THE PROCEDURE OF GETTING PLASMA AND SERUM

Overview. Blood consists of the formed elements (40-45%) suspended in a fluid called plasma(55-60%). Blood and plasma have slightly higher specific gravities than water, primarily because of the blood cells and proteins, but the slight difference is usually disregarded when estimating blood or plasma volumes based on body weight.)

The formed elements of the blood include erythrocytes (red blood cells)(4.5-6 mln/mm3), leukocytes (white blood cells)(4000-20000/mm3), and thrombocytes (also called platelets) (200000 - 400000/mm3).

Plasma – fluid portion of the blood, is about 92% water and 8% other substances. The main organic substances are: proteins (albumin – 4%, globulins – 2.8%, fibrinogen – 0.4%), carbohydrates (glucose – 0.08-0.12%), lipids (cholesterine – 0.7%). The inorganic micro- and macro-elements are distinguished: NaCl (0.8%), Ca (9-11 mg%), P (3.00 – 4.00 mg%). There are also some anions: HCO3 -, HPO4. The microelements are Co, Ni, Cu, Mg, Mn, etc.)

Objectives. To become familiar with the procedure of getting plasma and serum in order to complete subsequent lessons with the following objectives.

Equipment. Laboratory animal (rabbit, guinea pig, rat, mouse), blood needles, cotton wool, 96 ° alcohol, anticoagulant (5% sodium citrate, heparin), glass test tubes, centrifuge tubes, centrifuge.

Procedure. The following includes detailed instructions for the procedure of getting plasma and serum listed below.

Several anticoagulants used to prevent blood clotting outside the body do so by binding calcium ions. These include sodium citrate, potassium citrate, ammonium citrate, and ethylenediaminetetraacetic acid (EDTA). EDTA is usually in the form of a sodium or potassium salt. We use heparin as anticoagulant.

First of all anticoagulant is poured into a test tube. Animals blood is collected out of the vein into a test tube. When a portion of blood is treated with an anticoagulant, it prevents clotting and permitted to stand in a tube undisturbed. The tubes, containing blood with an anticoagulant, are centrifuged during 10 min under 3000 qn\1 min. A machine (centrifuge) separates plasma from red blood cells and we will get the formed elements and plasma. The formed elements erythrocytes, leukocytes, thrombocytes- all the cells gradually settle to the bottom of the tube, gathered together like sand. Plasma fluid portion of the blood, a strawcolored fluid above the formed elements.

To get serum animals blood is collected out of the vein into a test tube without an anticoagulant. When blood is drawn into a test tube without an anticoagulant and leaves for some time (2 hours) under 36-37°C, we will get clotting and serum.

Serum. When blood is allowed to clot, the cells are trapped in a meshwork of clotting proteins, leaving a yellow fluid, the serum. Essentially, serum is plasma minus the plasma proteins responsible for producing the clot. Serum from that animal can then be injected into an animal susceptible to the same disease to provide passive protection for as long as the antibodies remain in the susceptible animal. This provides merely temporary immunity.

Clotting may also be stimulated outside of the body when a foreign surface, such as the glass surface of a test tube (when blood is drawn into an untreated glass tube), induces the same reactions as exposure to collagen.

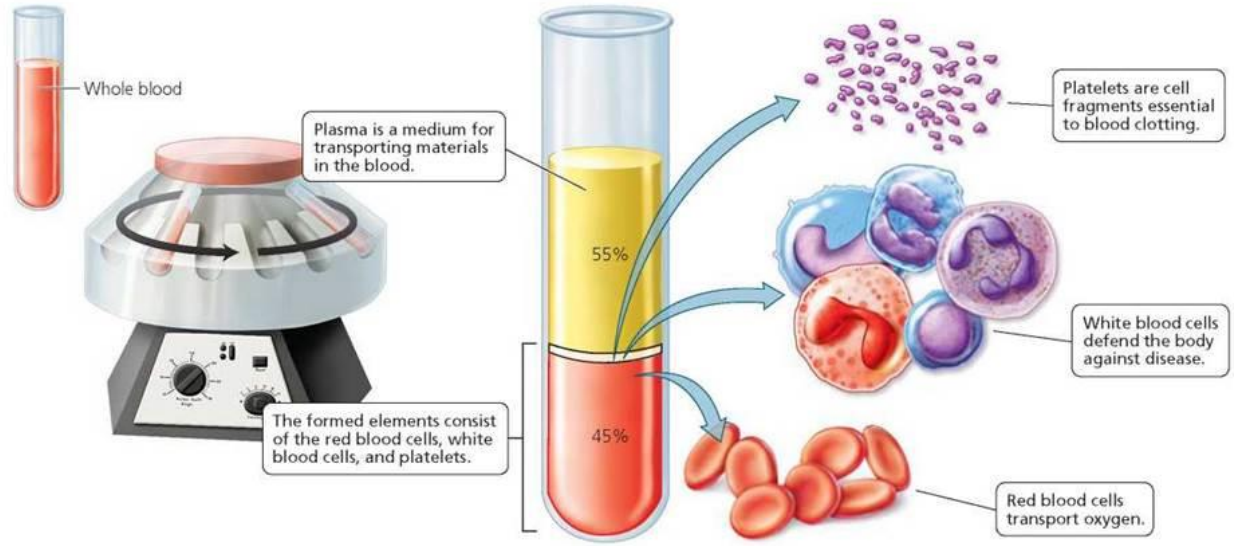


Figure 3. The procedure of getting plasma

Notice. Here and below figures are taken from Rowen D. Frandson, W. Lee Wilke, Anna Dee Fails. Anatomy and Physiology of Farm Animals. 7th ed. Publisher: A John Wiley & Sons, Inc., Publication, 2015. 536 pages

Research results. In results students describe the differences in procedure of getting plasma and serum.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 3
HEMOLYSIS OF ERYTHROCYTES

Overview. Hemolysis is the breakdown of erythrocytes and the release of hemoglobin. A lot of factors can cause so much hemolysis that the hemoglobin in plasma produces a reddish color, and the condition is called hemoglobinemia. The hemoglobin can then be excreted in the urine, and this is termed hemoglobinuria (red water).

There are other types of hemolysis.

Erythrocytes and other blood formed elements are destroyed in the hypotonic solution. This phenomenon is called osmotic hemolysis.

Chemical hemolysis is caused by the action of chemical agents (alcohol, acids and bases).

Physical hemolysis occurs in action of physical factors (temperature, mechanical agents, ultraviolet and other rays). Hemolysis can also be produced in a blood sample by physically disrupting the erythrocytes (e.g., forceful and rapid expulsion of blood from a syringe).

Biological hemolysis is observed in transfusion of the incompatible blood, under the action of poisons of snakes or in presence of Bacterial toxins, blood parasites.

Physiological hemolysis is the result of the destroy of old erythrocytes (the life of erythrocytes is 120 days).

Objectives. To become familiar with the procedure of chemical hemolysis of erythrocytes in order to complete subsequent lessons with the following objectives.

Equipment. Blood with anticoagulant (5% sodium citrate, heparin), glass test tubes, tripod, distilled water, 0.1% hydrochloric acid solution, ammonia, 0.9% solution of NaCl (the isotonic solution), 0.3 % solution of NaCl (the hypotonic solution).

Procedure. The following includes detailed instructions for the procedure of chemical hemolysis of erythrocytes listed below.

Take 5 test tubes and put them in a tripod. Then add in each test tube 5 ml of each solution, described in equipment. Then drop the blood to each tube and mix the contents. Consider the contents of each tube - the color of the solution, the color and consistency of the precipitate, if it was formed.

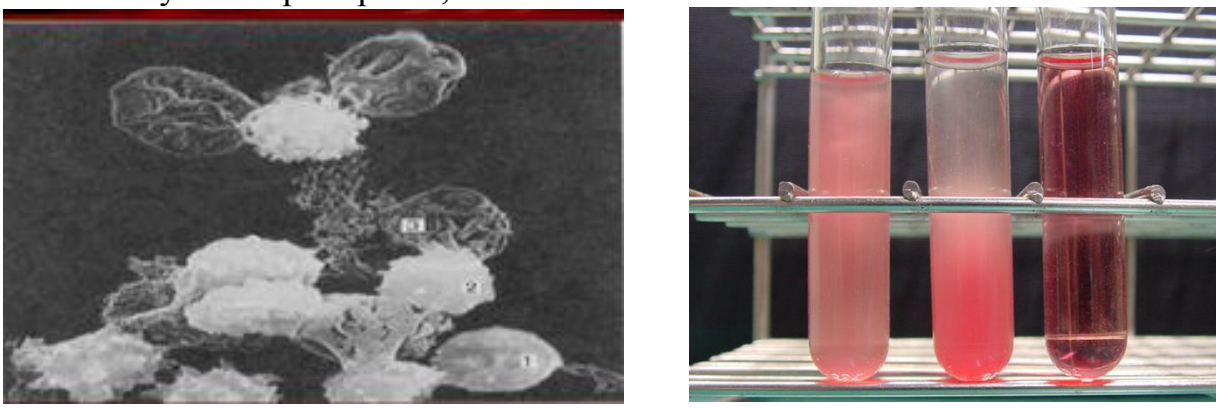


Figure 4. The chemical hemolysis of erythrocytes

Research results. In results students describe the differences in procedure of chemical hemolysis of erythrocytes.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 4
DETERMINATION OF OSMOTIC RESISTANCE OF ERYTHROCYTES

*Overview.*Osmotic pressure is the pressure by which the molecules of dissolved substance act on the unit surface of the semipermeable membrane, or the force, which provides the entering of dissolvent through semi permeable membrane from the

medium with less concentration to the medium with the high concentration. The osmotic pressure is conditioned mainly by inorganic substances (salts).

Osmosis (the Passive Transport of Water) – the diffusion of water across a semi-permeable membrane. Plasma membrane permeable to water but not to solute. Solute = dissolved particle. Water moves from solution with lower concentration of dissolved particles to solution with higher concentration of dissolved particles. Osmotic potential – is the total of all dissolved particles

Isotonic Solution: Solute concentration equal to that of cell. No net water movement.Those solutions which have the osmotic pressure like blood are called isotonic. The 0.9% solution of NaCl is called physiological solution. Other physiological solutions are Ringer's solution (for cold-blooded animals),Locke-Ringer's solution and Tyrode's solution (for warmblooded animals).

Hypotonic Solution: Solute concentration lower than cell. Less dissolved particles outside of cell than inside of cell. Hypo = less, under (think hypodermic, hypothermia); Tonic = dissolved particles. Water moves into cell from solution. Cell expands (and may burst).The solutions, which osmotic pressure is less than that of blood are called hypotonic once.

Hypertonic Solution: Solute concentration higher than cell. The solutions, which osmotic pressure is more than that of the blood are called hypertonic once.

Erythrocytes and other blood formed elements are destroyed in the hypotonic solution. This phenomenon is called **osmotic hemolysis**. In hypertonic solution the erythrocytes are squeezed, which is called **plasmolysis**.

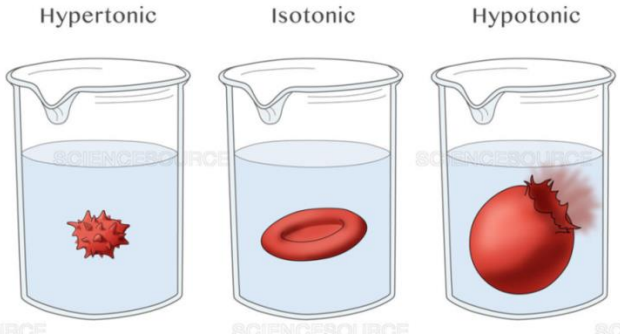


Figure 5. Plasmolysis (left), absence (middle) and osmotic (wright) hemolysis. of erythrocytes

Objectives. To become familiar with the procedure of determination of osmotic resistance of erythrocytes in order to complete subsequent lessons with the following objectives.

Equipment. Blood with anticoagulant (5% sodium citrate, heparin), glass test tubes, tripod, 0.9% solution of NaCl (the isotonic solution), 0.8 %, 0.7%, 0.6%, 0.5 %, 0.4%, 0.3%, 0.2 %, 0.1% solutions of NaCl (the hypotonic solutions).

Procedure. The following includes detailed instructions for the procedure of determination of osmotic resistance of erythrocytes listed below.

Determination of osmotic resistance of erythrocytes is carried out in solution NaCl from 0,9 % untill 0,1 %. Take 9 test tubes and put them in a tripod. Then add in each test tube 5 ml of each solution, described in equipment. Then drop the blood to

each tube and mix the contents. Consider the contents of each tube - the color of the solution, the color and consistency of the precipitate, if it was formed.

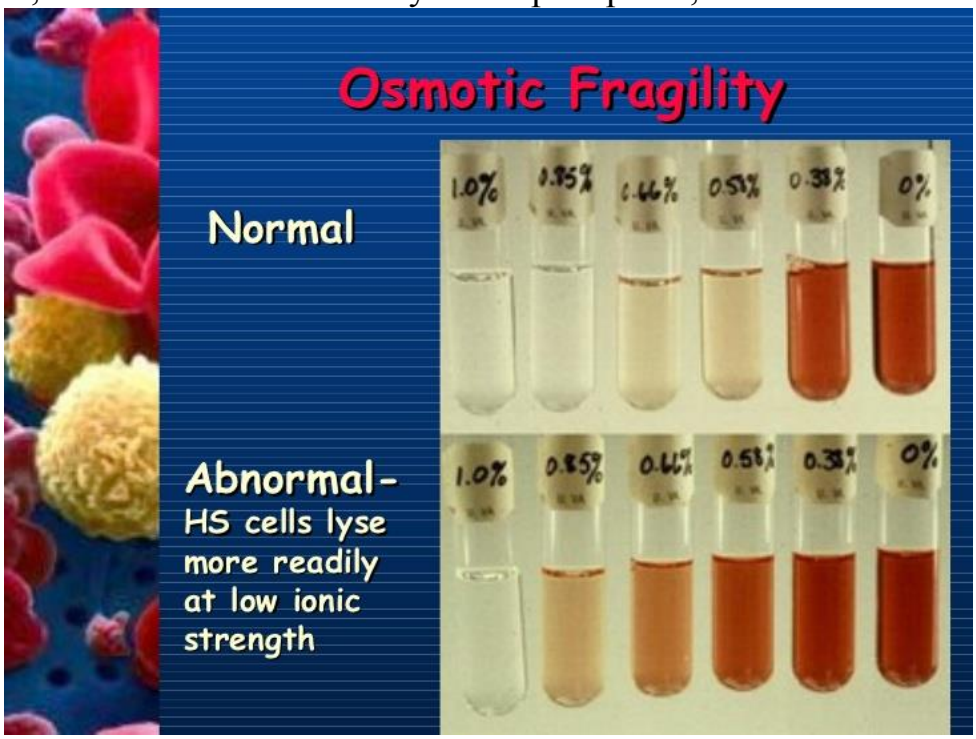


Figure 6. Osmotic resistance of erythrocytes

Min. osmotic resistance of erythrocytes –NaCl (0,44–0,75 %), hemolysis starts.

Max. osmotic resistance of erythrocytes –NaCl (0,32-0,44 %), hemolysis ends.

Osmotic resistance of erythrocytes decreases with famine, poisoning, chloroform, fatigue, hemolytic anemia, bleeding, weakened bone marrow activity.

Osmotic resistance of erythrocytes increases during pregnancy, after extensive blood loss, with post-hemorrhagic anemia, vaccinations, infectious diseases, increased bone marrow activity.

Osmotic resistance of erythrocytes is test of function of bone marrow, of erythrocyte formation (erythropoiesis).

Research results. In results students describe the differences in procedure of determination of osmotic resistance of erythrocytes.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 5
ERYTHROCYTE SEDIMENTATION RATE (ESR)

Overview. An erythrocyte sedimentation rate (ESR) is a type of blood test that measures how quickly erythrocytes (red blood cells) settle at the bottom of a test tube that contains a blood sample. Normally, red blood cells settle relatively slowly. A faster-than-normal rate may indicate inflammation in the body. Inflammation is part of your immune response system. It can be a reaction to an infection or injury. Inflammation may also be a sign of a chronic disease, an immune disorder, or other medical condition.

ESR can be increased in different inflammatory processes, cancer-genesis, during pregnancy, stress, allergy, etc. The value of ESR depends on the properties of plasma, primarily on the proteins (albumins, globulins and fibrinogen). Globulins and fibrinogen decrease the negative charge on the surface of erythrocytes, providing the interaction of these formed elements and due to it the increase of ESR. In contrast, the albumins decrease ESR. Decrease ESR also is observed in newborns, fatigue, jaundice. So, ESR depends on albumin/globulin coefficient. Sometimes in anemia ESR also can be accelerated.

Objectives. To become familiar with the procedure of erythrocyte sedimentation rate in laboratory animals in order to complete subsequent lessons with the following objectives.

Equipment. Laboratory animal (rabbit, guinea pig, rat, mouse), blood needles, cotton wool, 96 ° alcohol, 5% sodium citrate, glass test tubes (capillary tubes).

Procedure. The following includes detailed instructions for the procedure of erythrocyte sedimentation rate listed below.

Blood to which 5% sodium citrate has been added will settle in a certain time, the erythrocytes will be deposited as a sediment. Blood is collected to mark «P», 5% sodium citrate is collected twice to mark «K» and mixed. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour, is the ESR.

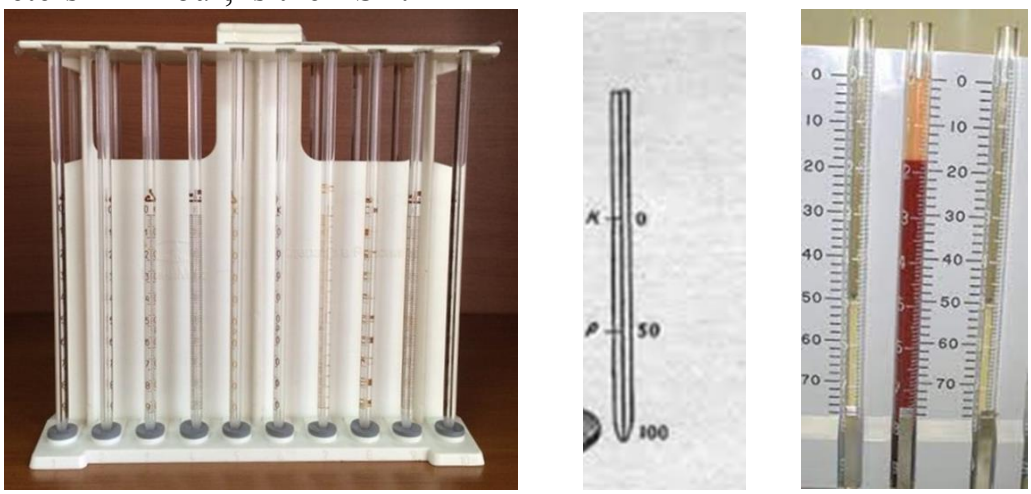


Figure 7. The procedure of erythrocyte sedimentation rate

In norm ESR in males is 2-9 mm/h; in females 6-15 mm/h, in newborns 0.5-1.0 mm/h. The estimation of ESR has a prognostic significance.

Research results. In results students describe the differences in procedure of erythrocyte sedimentation rate.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 6
THE HEMATOCRIT

Overview. The hematocrit (packed cell volume) is the percentage of the volume of a blood sample occupied by cells, 40-45%). The hematocrit (Ht or HCT), also known by several other names, is the volume percentage (vol%) of red blood cells (RBC) in blood, measured as part of a blood test. It is a part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count and platelet count.

Because the purpose of red blood cells is to transfer oxygen from the lungs to body tissues, a blood sample's hematocrit—the red blood cell volume percentage—can become a point of reference of its capability of delivering oxygen. Hematocrit levels that are too high or too low can indicate a blood disorder, dehydration, or other medical conditions. An abnormally low hematocrit may suggest anemia, a decrease in the total amount of red blood cells, while an abnormally high hematocrit is called polycythemia. Both are potentially life-threatening disorders.

Objectives. To become familiar with the procedure of hematocrit determinations in laboratory animals in order to complete subsequent lessons with the following objectives.

Equipment. Laboratory animal (rabbit, guinea pig, rat, mouse), blood needles, cotton wool, 96 ° alcohol, anticoagulant (5% sodium citrate, heparin), hematocrit tubes, centrifuge machine.

Procedure. The following includes detailed instructions for the procedure of hematocrit determinations listed below.

Routine hematocrit determinations require a glass tube treated to inhibit blood clotting (hematocrit tubes). The original method for hematocrit measurement is the macrohematocrit (Wintrobe hematocrit tube) method. It uses a narrow glass tube and a centrifuge machine. Hematocrit tubes containing blood are centrifuged until the blood cells are packed in the lower end of the tube. The centrifuge separates the blood into three main layers: red blood cells, white blood cells, and platelets, in that order from the bottom up. At the very top, there's a small layer of blood plasma. The macrohematocrit method carries the risk of measuring trapped plasma as part of the red blood cell layer, which erroneously elevates the hematocrit percentage.

Figure 8. The procedure of hematocrit determinations, layering of blood components in an anticoagulated and centrifuged blood sample.

Hematocrits typically range from 35 to 45 for most mammalian species and are generally considered to be an indicator of the total erythrocyte count. The measurement depends on the number and size of red blood cells. It is normally 40.7–50.3% for males and 36.1–44.3% for females.

A newer method is the microhematocrit method, which uses a capillary tube instead of a narrow glass tube and requires a smaller quantity of blood and less time. The microhematocrit method traps less plasma because the diameter of the capillary tube is smaller than the Wintrobe hematocrit tube. So the percentage of hematocrit is more accurate in the microhematocrit method than in the macrohematocrit method.

Both procedures require the technician to manually measure the length of the layers. The technician calculates hematocrit as the length of the packed red blood cell layer divided by the length of total cells and plasma. This ratio is then multiplied by 100 to give a certain percentage.

Research results. In results students describe the differences in procedure of hematocrit determinations.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 7
DETERMINATION OF HEMOGLOBIN LEVEL. SAHLI´S METHOD

Overview. The hemoglobin test (count) is a common blood test that measures your hemoglobin count. It is typically measured as part of a complete blood cell count, along with hematocrit measurement.

The clinician can then ascertain the hemoglobin concentration by reading from the calibration tube. Although this is one of the most common methods for estimating hemoglobin in developing countries and is relatively simple and inexpensive, the results are not always precise. For example, there is often inter-observer variability, and it's also highly prone to errors due to manual pipetting.3

Objectives. To become familiar with the procedure of determination of hemoglobin level in blood in laboratory animals in order to complete subsequent lessons with the following objectives.

Equipment. Laboratory animal (rabbit, guinea pig, rat, mouse), blood needles, cotton wool, 96 ° alcohol, anticoagulant (5% sodium citrate, heparin), 0.1 % solution of hydrochloric acid, Sahli's hemoglobinometer, pipette, distiller water, hemoglobin tube.

Procedure. The following includes detailed instructions for the procedure of determination of hemoglobin level in blood listed below.

Sahli's hemoglobinometer is a manual device that contains a hemoglobin tube, pipette, and stirrer, as well as a comparator. First of all we pour in hydrochloric acid into a hemoglobin tube to mark 10. Next we collect the appropriate amount of blood to mark 0.02 in pipette and pour it into bottom of the tube. Hydrochloric acid converts hemoglobin to acid hematin, which is then diluted by dropping of distiller water until the color of the solution matches that of the comparator block.

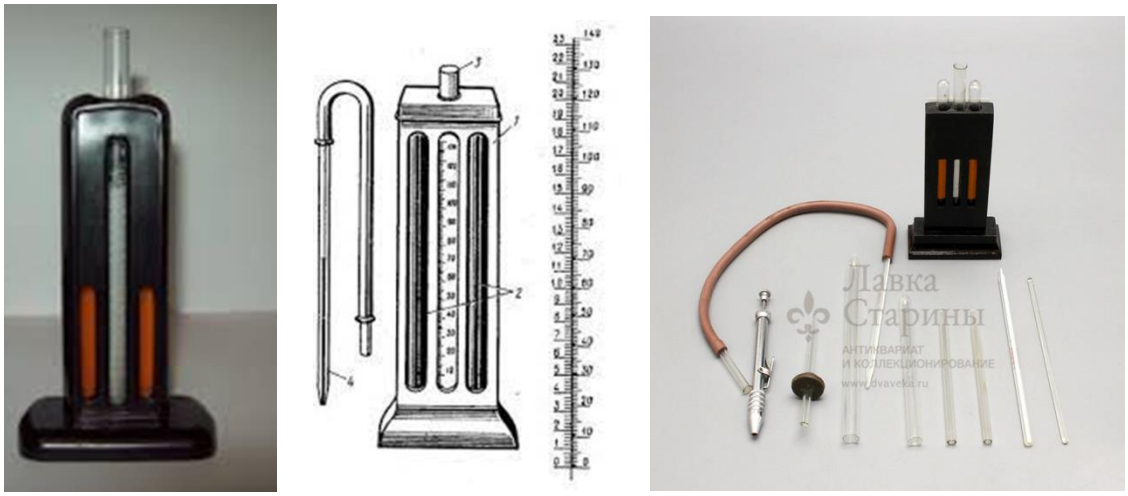


Figure 9. Procedure of determination of hemoglobin level in blood. Sahli's method

Research results. In results students describe the differences in procedure of determination of hemoglobin level in blood.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 8
RED BLOOD CELL COUNT

Overview. A red blood cell (RBC) count measures the number of red blood cells, also known as erythrocytes, in blood. Red blood cells carry oxygen from your lungs to every cell in your body. Your cells need oxygen to grow, reproduce, and stay healthy. An RBC count that is higher or lower than normal is often the first sign of an illness.

Objectives. To become familiar with the procedure of technique of red blood cell count in laboratory animals in order to complete subsequent lessons with the following objectives.

Equipment. Laboratory animal (rabbit, guinea pig, rat, mouse), blood needles, cotton wool, 96 ° alcohol, anticoagulant (5% sodium citrate, heparin), special glass pipettes for RBC, hemocytometer, glass cover, 3 % NaCl, light microscope.

Procedure. The following includes detailed instructions for the procedure of red blood cell count listed below.

First of all we collect drop of the blood from the animal. We can also use blood with an anticoagulant for this purpose. Next we collect the appropriate amount of blood to mark 0.5 in special glass pipette for RBC. Next we collect the appropriate amount of 3 % NaCl to mark 101 in the same special glass pipette for RBC. Next we close the same special glass pipette for RBC and shake it for some minutes.

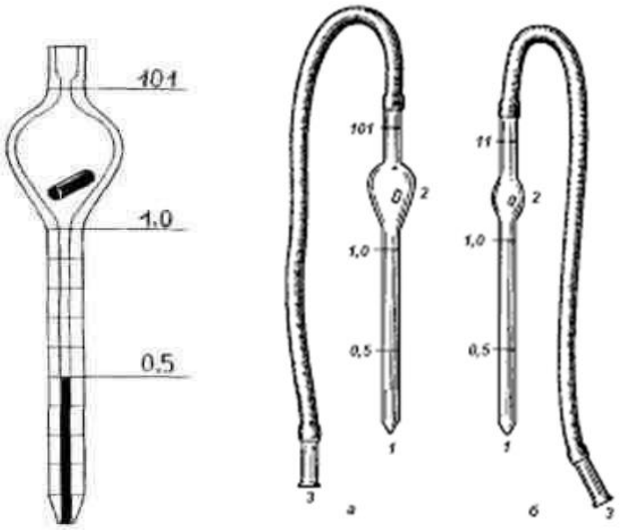
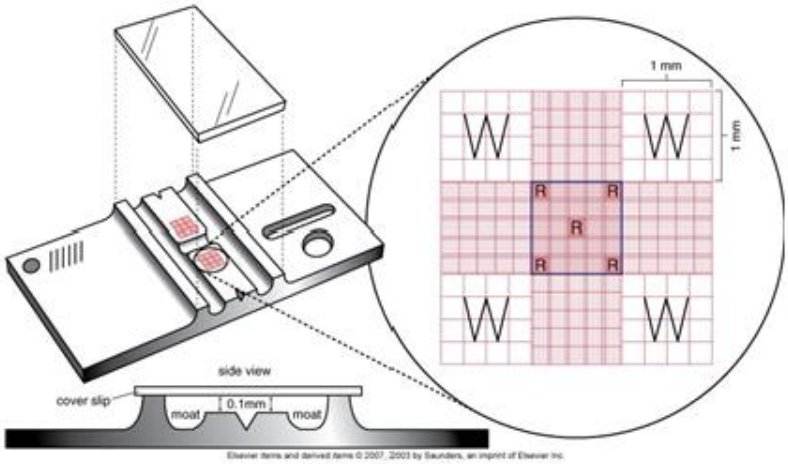


Figure 10. Procedure of mixing blood with 3 % NaCl

Manual cell counts (RBC, nucleated cells or platelets) are performed using a hemocytometer. Hemocytometer – is a device used in manual blood cell counts. We use glass cover on hemocytometer. To count cells using a hemocytometer, add 15-20µl of cell suspension between the hemocytometer and cover glass using a P-20 Pipetman. The hemocytometer with blood we put under light microscope.

The hemocytometer is divideded into 9 major squares of 1mm x 1mm size. The four coner squares (identified by the red square) are further subdivided into 4 x 4 grids. The height of the chamber formed with the cover glass is 0.1 mm, so a 1 mm x 1 mm x 0.1 mm chamber has a volume of 0.1 mm3 or 10-4 ml.



- Hemocytometer
  - A device used in manual blood cell counts
    - Sections to count
      - WBC (W)
      - RBC (R)
      - Platelets (whole plate)

Figure 11. The hemocytometer.

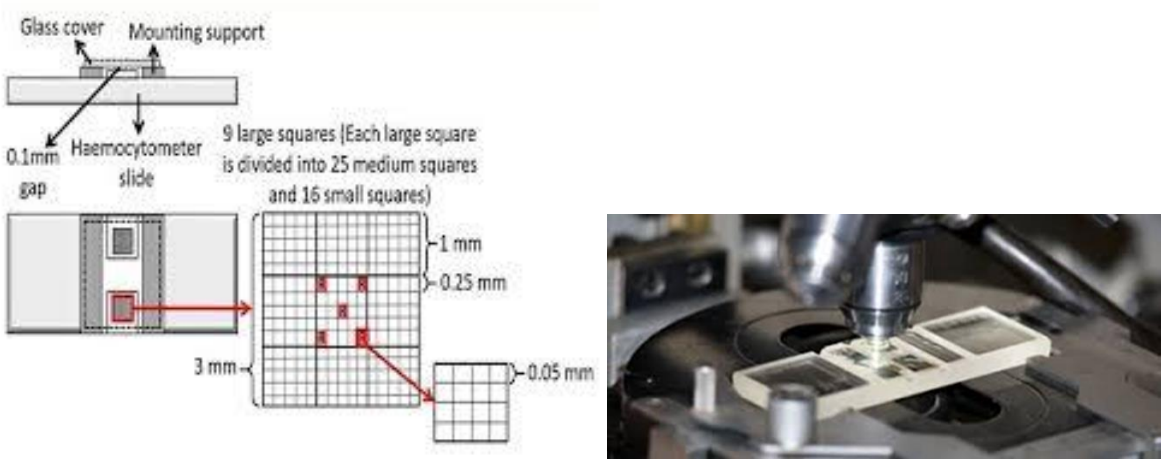


Figure 12. The hemocytometer. Technique of red blood cell count.

Red blood cell we count under light microscope (10x, 40x) in 5 big divided squares (fig. 13 middle). One big divided square consists of 16 small undivided squares (fig. 13 left). So, in general we should count cells in 80 small undivided squares. RULE – count erythrocytes, which lie inside the small squares and on the upper and left lines. Erythrocytes lying on the right and lower side do not count.

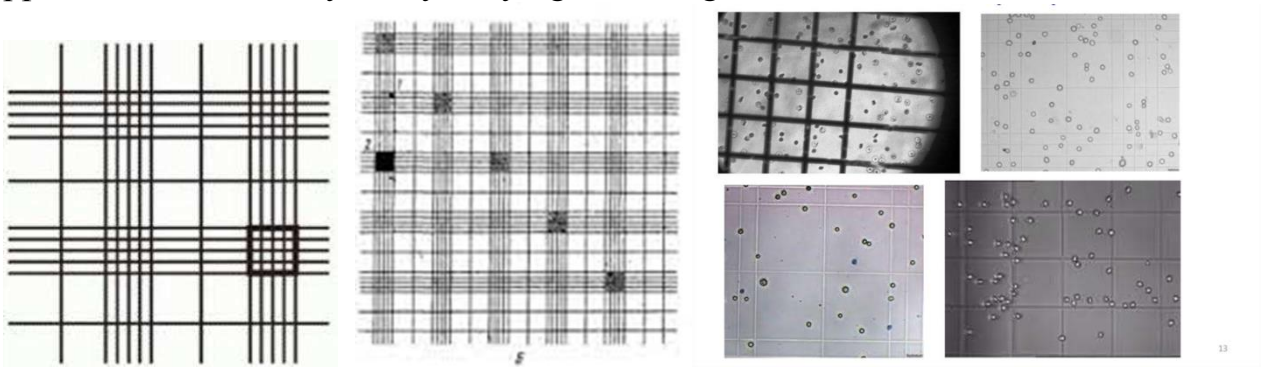


Figure 13. Technique of red blood cell count in the hemocytometer. Red blood cells under light microscope (10x, 40x)

After we finish count we use formula

$$x = \frac{\mathrm{a} \cdot 4000 \cdot 200}{80}, \qquad x = a \cdot 10000$$

When finished, spray the hemocytometer and cover slip with 70% ethanol to kill the cells. Wash both with deionized water and wipe dry with a Kimwipe. Wrap in a clean Kimwipe and return to the storage box. Note, the cover slips for the hemocytomer are made of a special thicker/flatter glass. Please try to avoid breaking or losing it. If you do, reorder hemocytomer cover slips, not regular cover slips.

Research results. In results students describe the differences in procedure of red blood cell count.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 9
WHITE BLOOD CELL COUNT

Overview. A white blood cell (WBC) count measures the number of white blood cells, also known as leukocytes, in blood. An WBC count that is higher or lower than normal is often the first sign of an illness.

Objectives. To become familiar with the procedure of technique of white blood cell count in laboratory animals in order to complete subsequent lessons with the following objectives.

Equipment. Laboratory animal (rabbit, guinea pig, rat, mouse), blood needles, cotton wool, 96 ° alcohol, anticoagulant (5% sodium citrate, heparin), special glass pipettes for WBC, hemocytometer, glass cover, Tiurks solution (2 % acetic acid+blue solution), light microscope.

Procedure. The following includes detailed instructions for the procedure of white blood cell count listed below.

The procedure of white blood cell count is similar to red blood cell count. First of all we collect drop of the blood from the animal. We can also use blood with an anticoagulant for this purpose. Next we collect the appropriate amount of blood to mark 0.5 in special glass pipette for WBC. Next we collect the appropriate amount of Tiurks solution to mark 1.1 in the same special glass pipette for WBC. Next we close the same special glass pipette for WBC and shake it for some minutes.

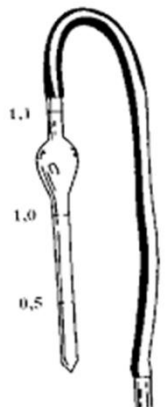


Figure 14. Special glass pipettes for WBC

Manual cell counts (WBC) are performed using a hemocytometer. We use glass cover on hemocytometer. To count cells using a hemocytometer, add 15-20µl of cell suspension between the hemocytometer and cover glass using a P-20 Pipetman. The hemocytometer with blood we put under light microscope.

The goal is to have roughly 100-200 cells/square. Count the number of cells in all four outer squares divide by four (the mean number of cells/square). The number of cells per square x 104 = the number of cells/ml of suspension. This protocol works well for either adherent mammalian cells that have been trypsinized or for suspension cells including Sf9 insect cells. Red blood cells are typically too small and numerous for this protocol and utilize the middle square instead.

White blood cell we count under light microscope (10x, 5x) in 100 big undivided squares (fig. 15 left).

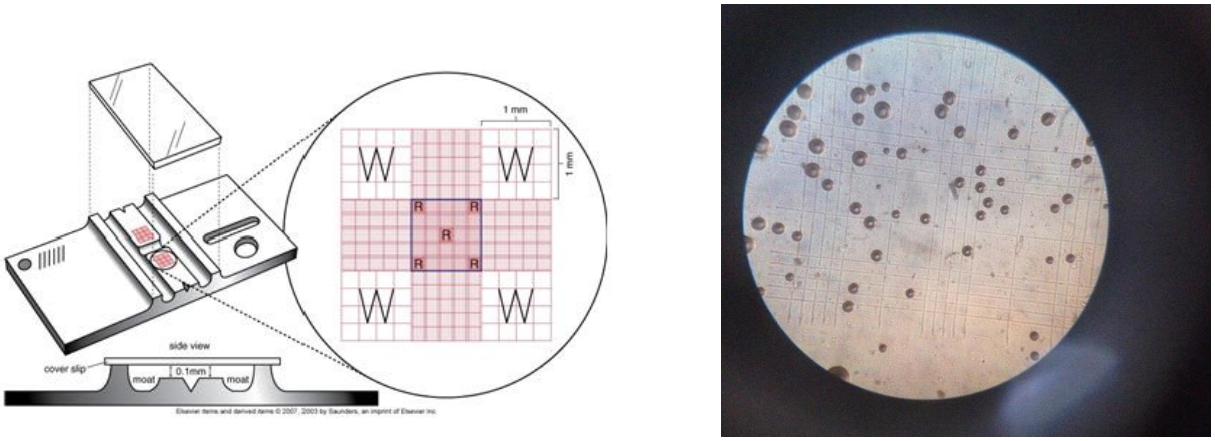


Figure 15. Technique of white blood cell count in the hemocytometer. White blood cells under light microscope (10x, 5x)

After we finish count, we use formula

$$x = \frac{a \cdot 250 \cdot 20}{100}, \text{ або } x = a \cdot 50$$

Research results. In results students describe the differences in procedure of white blood cell count.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 10
DIFFERENTIAL WHITE BLOOD CELL COUNTS

Overview. Differential counts indicate the percentage of each type of white cell in the blood sample. The various types of leukocytes have different functions and respond differently to various types of infections or diseases, so differential counts can be useful for diagnosis. Differential counts taken over time can also be used to evaluate the response of an animal to infection or disease.

Objectives. To become familiar with the procedure of differential white blood cell counts in laboratory animals in order to complete subsequent lessons with the following objectives.

Equipment. Laboratory animal (rabbit, guinea pig, rat, mouse), blood needles, cotton wool, 96 ° alcohol, Wright's stain, glass slide, light microscope.

Procedure. The following includes detailed instructions for the procedure of differential white blood cell counts listed below.

A differential count is made by spreading a drop of whole blood thinly on a glass slide to form a blood smear.

The smear is dried and stained with a blood stain, such as Wright's stain. After staining is complete, the slide is examined with a microscope and the number of white cells of each kind is tabulated until a predetermined total number of white cells have been counted. The number counted is usually a multiple of 100, and the percentage of each leukocyte type observed in a given sample of blood is called the

differential leukocyte count or differential white cell count. In reference laboratories, both total red and white cell counts are semiautomatically determined by sophisticated laboratory equipment.

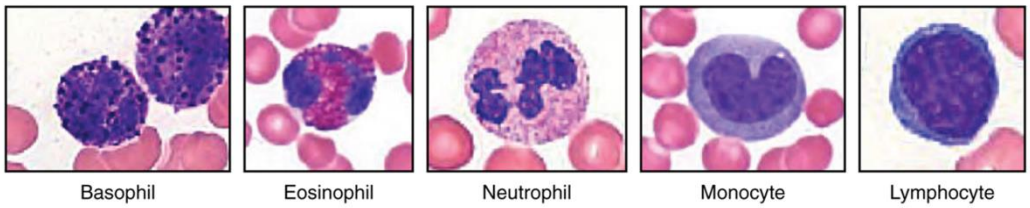


Figure 16. Differential white blood cell

The appearance of platelets in a stained smear may be considerably different from their actual appearance in circulating blood, where they are oval disks. In smears they may appear as circular disks, star-shaped fragments, or clumps of irregular shape.

Research results. In results students describe the differences in procedure of differential white blood cell counts.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 11
DETERMINATION OF BLOOD GROUPS

Overview. Medical practice is often faced with the need to replace the blood lost in hemorrhages, certain cases of poisoning, chronic infection as well as for other medical indications. In the past the attempts to transfuse blood not infrequently caused severe reactions, even lethal.

Hemagglutination is clumping of erythrocytes. Erythrocytes have membrane proteins that act as antigens, and agglutination results if erythrocytes with specific antigens are added to a solution containing appropriate antibodies.

Blood typing is a general term describing the procedures used to identify the antigen or antigens in a given blood sample. Clumping may occur during transfusions of blood within the same species, such as between humans, if blood of the wrong type (i.e., containing the wrong combination of antigens) is used. At least seven blood types have been identifi ed in horses, and it is desirable to ensure that there is adequate matching of types before attempting transfusions between horses. Blood typing has also been done in cattle and dogs, but usually a single transfusion can be done between two cattle or between two dogs with little diffi culty. However, repeated transfusions can be problematic, because the initial transfusion may provide unmatched antigens that stimulate the formation of antibodies. Blood types are inherited in all species, and they are often used to establish and monitor pedigrees.

In 1901 K.Landsteiner and in 1903 J. Jansky found that **erythrocytes glue together when the blood of different persons is mixed. This phenomenon known as *agglutination*** depends on the presence of erythrocytes of *agglutinable factors* or *agglutinogens* A *and B* on the membrane. In erythrocytes they can be found to occur either separately or together or be absent at all. It has simultaneously been established

that plasma contains *agglutinating agents*, which agglutinate erythrocytes. They are called *agglutinins* α *and* β. Agglutination of erythrocytes occurs when agglutinogens of the *donor* are combined with the same agglutinins of the *recipient*.

Hence, the blood of each individual has dissimilar agglutinogen and agglutinin. The human groups are *four (in the ABO system)* according to four combinations of agglutinogens and agglutinins. They are designated as follows: *I(0)*-α,β*; II(A)-A,*β*; III(B)-B,*α*; IV(AB)-AB. Agglutinogen A* has more than 10 variants. The difference between them is that *A1* is the strongest, while *A2–A7* and other variants have weak agglutination properties. For this reason, blood of such individuals can be erroneously included in group I.

Agglutinogen B also has a few variants which activity diminishes in the order of their numeration. Blood groups are inherited according to genetic principles and remain unchanged throughout life. Individuals belonging to group I can be transfused by the blood of the same group. Blood group I can be transfused to individuals of any blood group. Persons with blood group I are known as *universal donors*. But 10-20% of individuals with blood group I contain *anti-A* and *anti-B* agglutinins, therefore individuals with blood group I, containing *anti-A* and *anti-B* agglutinins are considered as *"hazardous" universal donors*. Individuals with blood group IV can be transfused by the blood of all groups and are known as *universal recipients*. Transfusion of this blood to individuals with other blood groups causes severe reactions (Table 2).

Table 2.

The agglutination after mixing of serum and erythrocytes of different blood groups.

Groups of agglutinins	Groups of agglutinogens			
	I (0)	II A	III B	IV AB
I (αβ)	-	+	+	+
II (β)	-	-	+	+
III (α)	-	+	-	+
IV (0)	-	-	-	-

Blood group IV can be transfused to persons with the same blood group. Blood of persons belonging to groups II and III can be transfused to those with the identical group and to group IV individuals.

Principles of hemotransfusion. Two main principles of blood transfusion have been formulated. *1. Blood should be crossmatched so as to prevent combination of identical agglutinogens of the donor with identical agglutinins of the recipient. 2. The donor agglutinins are disregarded*; this is socalled *the rule of dilution which is used in transfusion of small quantities of blood.* They lose their activity in dilution. The agglutinins should be diluted *10-15 times*. Transfusion of incompatible blood may cause *hemotransfusion* (*clotting*) *shock* which often ends death. The mechanism underlying its development is that the agglutinated erythrocytes on destruction secrete their coagulation factors including thromboplastin. The latter causes intravascular clotting and blockade of the microcirculatory vessels in all organs and tissues by fibrin and platelet thrombi.

Study of the blood groups in different countries has shown their percentage distribution: group I – 40-50%; group II – 30-40%; group III – 10-20%; group IV – 5 %.

Objectives. To become familiar with the procedure of determination of blood groups in students in order to complete subsequent lessons with the following objectives.

Equipment. Blood needles, cotton wool, 96 ° alcohol, glass slide, serum II and serum III.

Procedure. The following includes detailed instructions for the procedure of determination of blood groups listed below.

A determination of blood groups is made by spreading a drop of serum II and serum III on glass slide. Next student drops blood on a glass slide to each serum, mixes and waits 5 minutes.

Absence of agglutination in both serums means that blood belongs to group 1; agglutination with serum III — blood group II; with serum II — to blood group III; presence of agglutination in both serums means that blood belongs to group IV.

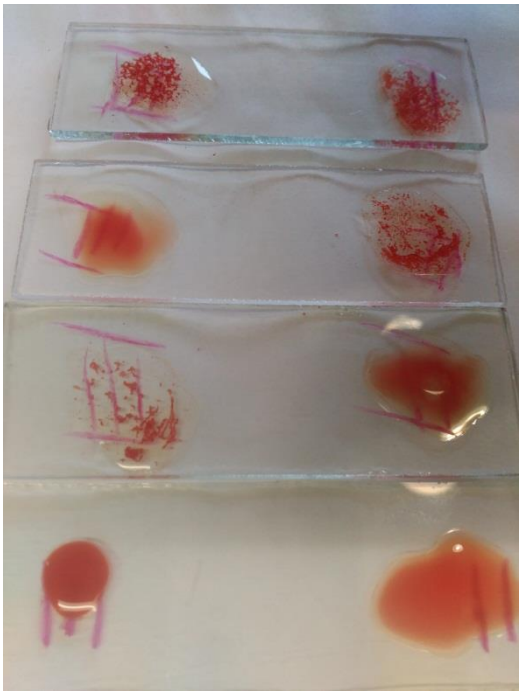


Figure 17. Determination of blood groups

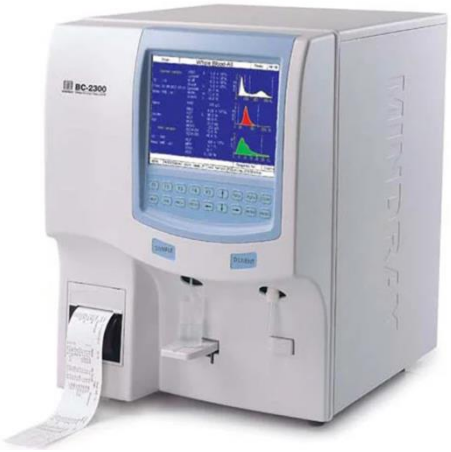
Research results. In results students describe the differences in procedure of determination of blood groups.

Conclusions. Conclusions are based on the analysis of the results of experimental studies.

LABORATORY CLASS 12
LABORATORY EXAMINATION OF BLOOD BY ANALYZER

Physicians rely upon laboratory analysis to obtain measurements of many constituents of the blood, information useful or necessary for the detection and recognition of disease. Adequate examination of the blood cells requires that a thin

film of blood be spread on a glass slide, stained with a special blood stain (Wright stain), and examined under the microscope. Individual red cells, white cells, and platelets are examined, and the relative proportions of the several classes of white cells are tabulated. The results may have important diagnostic implications. Several anticoagulants used to prevent blood clotting outside the body do so by binding calcium ions. These include sodium citrate, potassium citrate, ammonium citrate, and ethylenediaminetetraacetic acid (EDTA). EDTA is usually in the form of a sodium or potassium salt.



Biochemical and hematological analyzer of blood.

Tests, available by Biochemical and hematological analyzer of blood.
WBC — white blood cells;
RBC — red blood cells;
HGB — hemoglobin;
HTC — hematocrit;
MCV — mean corpuscular volume;
MCH — mean concentration hemoglobin;
MCHC — mean corpuscular hemoglobin concentration;
RDW — red cell distribution;
PLT — platelets;
MPV — mean platelets volume
PTC — Thrombocrit.
NEU (% and absolute) — Neutrophil;
LYMP (% and absolute) — Lymphocyte;
MONO (% and absolute) — Monocytes;
EO (% and absolute)— Eosinophils;
Baso (% and absolute) — Basophil.

An automated hematology analyzer where the hematocrit test is obtained as part of a complete blood count (CBC) laboratory test. The hematocrit is calculated indirectly from the average volume and the number of red blood cells.[2] The automated analyzer is the fastest method. However, automated analyzers may not be available in areas where resources are low (Osmotic resistance of erythrocytes and Erythrocyte sedimentation rate).

TESTS

1. Liquid which consists of the formed elements (40-45%) suspended in plasma (55-60%) is called:

A.Lymph,
B.Serum,
C.Interstitial fluid,
D.Blood.

2. The formation and development of all formed elements of blood (erythrocytes, leukocytes, and platelets) is called:

A.Blood system,
B.pluripotent stem cells,
C. Hematopoiesis,
D.creator connections.

3.Fluid portion of the blood without the plasma proteins is called:

A.Blood volume,
B.Serum,
C.Osmolality,
D.Plasma.

4.The relative constancy of internal medium of the organism is called:

A.Homeostasis,
B.Osmotic pressure,
C.oncotic pressure,
D.the viscosity.

5.A typical pH range for blood is:

A.7.35 to 7.45,
B.7.00 to 8.00,
C.4.35 to 6.35,
D.8.00 to 9.45.

6. Blood pH is supported at a constant level owing to the buffer systems, which quantity is:

A.2,
B.7,
C. 6,
D.4.

7.Carbon dioxide binds to α-amino groups of peptide chains to form carbaminohemoglobin, which is written as:

A.$HbCO_2$,
B.HbCO,
C.HbO_2,
D.MeHb.

8. Their primary function s of Eosinophils are:

A.damage of tissue,
B.independent movement to exit blood vessels,
C.the regulation of allergic responses,
D. the regulation of tissue responses to parasites.

9. The essential leukocytes that develop a specific immune response and immune surveillance are:

A.Monocytes,

B. Lymphocytes,

C. Basophils,

D. Eosinophils.

10.The formation of a clot or thrombus is called:

A.Hemostasis,

B.Platelet aggregation,

C.platelet plug,

D.Coagulation.

List of questions for current and summary (examination) knowledge control to the topic: physiology of the blood system

1. The concept of the blood system. The internal environment of the body (blood, interstitial fluid, lymph).
2. The functions of blood. Organs of blood keeping (conserving). Total blood volume in different animals.
3. The properties of blood.
4. The buffer systems. Regulation of acid-base balance.
5. The composition of blood.
6. The composition of Plasma. The types of plasma proteins.
7. The oncotic and osmotic pressure.
8. Formed Elements of Blood: Erythrocytes, structure and fuctions. Total erythrocyte counts.
9. Hemolysis, types of hemolysis.
10. Osmotic resistance of erythrocytes.
11. Erythrocytes sedimentation rate. Physiological effect. Diagnostics.
12. Forms of hemoglobin.
13. Formed Elements of Blood: Leukocytes. Functions of different types of leukocytes.
14. T-, B-Lymphocytes function and Specific Immune Response
15. Differential White Blood Cell Counts.
16. Formed Elements of Blood: Blood platelets (thrombocytes).
17. Hemostasis and Coagulation.
18. Blood groups and RH-factor.
19. Principles of hemotransfusion.
20. Hematopoiesis and its regulation.

BIBLIOGRAPHY

1.Animal physiology. The program of the discipline for the training of EQL specialists "Bachelor" in the direction of 6.110101 "Veterinary Medicine" in higher educational institutions of II-IV levels of accreditation of the Ministry of Agrarian Policy of Ukraine / [Mazurkevich A.Y., Karpovsky V.I., Trokaz V.O., Kladnytska L.V., Zhurenko O.V., Krivoruchko D.I, Kambur M.D., Nishchemenko M.P, Fedoruk R.S., Golovach P.I. etc.]. Kyiv: Agrarian Education, 2010. 26 pages.

2. Floron C. Faries, Jr., DVM, MS. Anatomy & Physiology of Animals. 2013. 84 p.

3. Gerard J. Tortora and Bryan H. Derrickson. Principles of Anatomy and Physiology. 11th ed. Publisher: Hoboken, N.J. : John Wiley & Sons, 2006. 512 pages.

4. Hafez E.S.E. and B. Hafez. Reproduction in Farm Animals. 7th ed. Philadelphia: Lippincott Williams & Wilkins, 2013. 509 pages.

5. James G. Cunningham, Bradley G. Klein. Textbook of veterinary physiology. 5th ed. Publisher: St. Louis: Saunders Elsevier, cop. 2013. 608 pages.

6. Neil R. Carlson, Melissa A. Birkett. Physiology of behavior. 12th edition. Publisher: Pearson India. 2017. 738 pages.

7. Pineda M.H., Dooley M.P. Veterinary Endrocrinology and Reproduction. 5th ed. Publisher: Iowa State Press, Ames, USA. 2003. 597 pages.

8. Physiology of farm animals: workshop (second edition, revised) / Mazurkevich A.Y., Trokoz V.O., Karpovsky V.I., Stepchenko L.M., Stoyanovsky V.G., Golovach P.I. etc. Kyiv: Center for Educational Literature, 2015. 240 pages

9. Physiology of agricultural animals: textbook: second edition, revised / [Mazurkevich A.Y, Trokoz V.O., Karpovsky V.I., etc.]; edited by A.Y. Mazurkevich, V.O Trokoz. Kyiv, 2013. 476 pages.

10 R. Michael Akers and D. Michael Denbow. Anatomy and physiology of domestic animals. 2nd ed. Publisher: John Wiley & Sons. Wiley-Blackwell. 2013. 684 pages.

11. Richard W. Hill, Gordon A. Wyse, and Margaret Anderson. Animal Physiology. 4th ed. Publisher: Oxford University Press. 2017. 1008 pages.

12. Richard W. Hill, Gordon A. Wyse, Amherst Margaret Anderson. Animal Physiology 3rd ed. Publisher: Sinauer Associates, Inc. Sunderland, Massachusetts. 2012. 985 pages.

13. Rodney A. Rhoades, Richard G. Pflanzer, Human Physiology. 4th ed. Publisher: Australia; Pacific Grove, Calif.: Thomson: Brooks/Cole. 2003. 1120 pages.

14. Rowen D. Frandson, W. Lee Wilke, Anna Dee Fails. Anatomy and Physiology of Farm Animals. 7th ed. Publisher: A John Wiley & Sons, Inc., Publication, 2015. 536 pages.

15. Ruth Lawson. Anatomy and Physiology of Animals. WikiBooks. 2015. 264 pages.

16. Thomas D. Pollard, William C. Earnshaw, Jennifer Lippincott-Schwartz, Graham T. Johnson. Cell Biology. 3rd ed. Publisher: Philadelphia, W.B. Saunders, Elsevier. 2016. 908 pages.

17. Walter F. Boron, Emile L. Boulpaep. Medical Physiology. 2nd ed. Publisher: Philadelphia, W.B. Saunders. 2009. 403 pages.
18. William O. Reece. Functional Anatomy and Physiology of Domestic Animals. 4th ed. Publisher: Wiley-Blackwell, Baltimore. 2009. 592 pages.
19. Victoria Aspinall, Melanie Cappello. Introduction to Veterinary Anatomy and Physiology Textbook. 3rd ed. Publisher: Elsevier Science & Technology. Oxford, United Kingdom. 2015. 288 pages.

Kolomiiets Iryna. Physiology of the blood system. Methodological instructions for laboratory classes of subject «Animal physiology» for foreign students of the speciality 221 «Veterinary medicine», branch of knowledge 21 «Veterinary Medicine». – Lviv : Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, 2022.– 26 p.