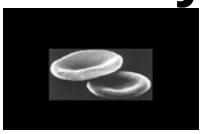
Blood Laboratory Exercises



Foreword:

It is well known that many diseases may be transmitted by human blood or other body fluids. Although no human blood will be used in the following exercises, in the interest of promoting general safety practices, the set of "Universal (Standard) Precautions" intended to minimize the risk of contamination from handling human samples will be utilized throughout the entire laboratory session. It is essential that you do not deviate from these simple instructions.

1. **Eating and drinking are strictly forbidden** in the laboratory area. **Wearing a lab coat is mandatory.**
2. **Protective disposable gloves must be worn at all times**. Skin cuts or abrasions must be covered by a waterproof dressing prior to the donning of gloves.
3. **Hands must be washed thoroughly** following the removal of gloves, and prior to leaving the laboratory area.
4. **All blood samples must be handled carefully** – avoid spilling or splashing.
5. **All used disposable materials (spectrophotometer cells, test tubes, gloves, etc.)** and disposable sharp objects (Pasteur pipettes) **must be placed in the cardboard box labeled with a "biohazard" symbol for disposal**.
6. Non-disposable items such as the hemoglobinometer chamber and clip, the hemocytometer must be rinsed with water and must remain on the bench top. The difquik stained slide (WBC differentiation) must be returned to the central bench.

In clinical practice, hematological information accumulated from a series of blood tests conducted on a small volume - even a single drop - of blood, can be of great diagnostic and prognostic value. Of the various kinds of simple blood tests available, three groups have been selected for this laboratory session:

Part A - Measurement of Erythrocyte Fragility (p.2).
Part B - Hemostatic Tests (p. 4)
Part C - Blood Cell Indices (p. 5).

It is recommended that the **entire** group participate in each of the tests. To derive the most benefit from the following exercises, students are asked to **review the information presented in class, as well as the appropriate chapters in the textbook** dealing with the various constituents of blood, their functional characteristics, and the regulatory factors that determine their numbers or concentrations.

NOTE: For reasons of safety, procedures for this lab have been modified. Some experimental procedures carried out on humans will be shown on video, while others will be performed using animal blood and using commercially-obtained simulated blood. Consequently, although instructions on drawing blood from human subjects are included so as to familiarize the student with the procedures, they should be disregarded for the actual experiments.

PART A - Measurement of Erythrocyte Fragility (OSMOTIC HEMOLYSIS)

Cell membranes are semipermeable barriers, and osmotic gradients are established between intracellular and extracellular fluids which can cause water to flow into and out of the cells. The amount of osmotic pressure depends upon the difference between the concentration of non-diffusible ions (osmols/L) on each side of the membrane. One osmol is equal to one mol of dissolved, non-diffusible, non-ionizable substance. If the substance completely ionizes into two ions, then one mol of the dissolved substance yields two osmols.

The intracellular fluid of erythrocytes is a solution of salts, glucose, protein, and hemoglobin. A 0.9% NaCl (MW = 58.5) solution is said to be **isotonic**: when blood cells reside in such a medium, the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water. When subjected to **hypertonic** media (e.g. 1.8% NaCl), the cells lose their normal biconcave disc shape, undergoing collapse (leading to **crenation**) due to the rapid osmotic efflux of water. 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.

In this experiment, we make use of the property that the osmotic fragility (or susceptibility to hemolysis) of erythrocytes is not uniform, and the number of cells undergoing hemolysis depends on the degree of hypotonicity of the extracellular medium. Thus, the percentage of erythrocytes which hemolyse when suspended in media of different hypotonicity (between isotonic saline and distilled water) varies. The concentration of liberated hemoglobin in each test medium is an index of the extent of osmotic hemolysis. Your problem is to examine experimentally the relationship between extent of hemolysis and osmolarity of the medium in which the erythrocytes are suspended.

Materials

- Oxalated (to prevent clotting) whole blood
- adjustable 20-200 μl range pipetman with corresponding disposable tips
- 6 labelled and capped centrifuge tubes containing 10ml of 0.90% (isotonic),0.60%, 0.50%, 0.40%, 0.30%, and 0.00 %(distilled water) NaCl solutions.
- Centrifuge
- Spectrophotometer with 6 disposable spectrophotometer cells
- Pasteur pipettes and bulb
- Marking pen
- kimwipes

Procedure

1. Mix the blood sample (by inverting the tube a few times) to obtain a homogeneous suspension of red blood cells.
2. Take 0.1ml (or 100 μl) of blood with the adjustable pipetman, wipe the outside of the yellow tip clean, then deliver the sample below the surface of the test solution in each of the centrifuge tubes.

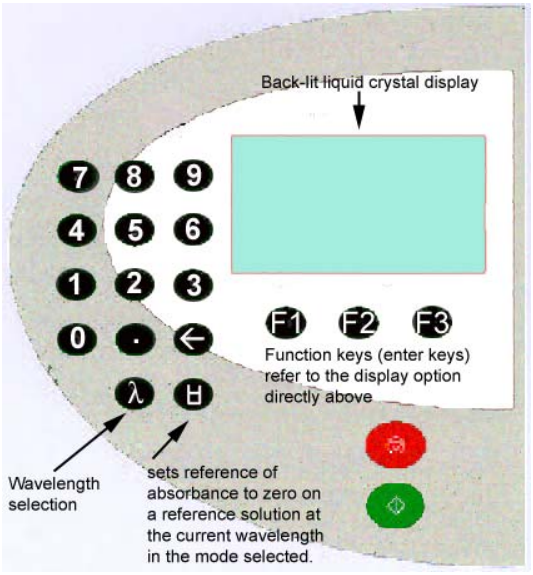
3. Cap the centrifuge tubes, and mix the contents by inverting the tubes several times.

4. Allow the samples to hemolyze for 20 minutes.

5. After 20 minutes, centrifuge all six tubes simultaneously for 10 minutes at maximal speed (about 1000 x g).

6. While centrifugation is going on, prepare the spectrophotometer.

Estimation of the concentration of solutions by spectrophotometry is possible because the quantity of light absorbed by the particles in a solution, or the optical density (O.D.), increases linearly with concentration. A light beam is shone through the solution in question and light absorption is detected using a photosensitive element which reads out **optical density** (or the log of its inverse, percent light "transmittance") on an appropriately calibrated meter scale.
Select the wavelength for optimum light absorption by hemoglobin by pressing on the "λ" and typing with the key pad: 540, press F3.



7. Using Pasteur pipettes with a rubber bulb and taking great care not to disturb the precipitated fraction, transfer about 3 ml of tube # 1 (0.9% NaCl) to a spectrophotometer cell (up to the etched mark); place it in the spectrophotometer and press on "U".
This provides the zero value for the calibration curve. (Assuming no hemolysis took place in the isotonic medium, the supernatant of tube #1 should absorb a minimum amount of light).

8. Transfer 3 ml of tube #6 (distilled water) to another spectrophotometer cell and read the displayed O.D. Assuming that complete hemolysis took place in the distilled water, this O.D. reading now corresponds to 100% hemolysis.

9. Transfer 3 ml from each of the remaining four test solutions into separate spectrophotometer cells and read off values of O.D.

10. Plot a standard curve on the results sheet and estimate the **percent of hemolysis** that took place in each medium on the basis of the recorded OD.

Results

a. Tabulate your results, and on the basis of your knowledge from class notes/textbook, calculate the osmolarity and the osmotic pressure (O.P.) in mm Hg of the NaCl solutions used.

b. Plot a graph of % hemolysis vs osmolarity of NaCl solution.

PART B - HEMOSTASIS (Video)

Hemostasis (literally - blood halting) depends on 3 interrelated and overlapping sets of events:

1–constriction of the blood vessels and formation of a platelet "plug"
2– blood clotting
3– clot retraction

I. Clotting Time Test

In order for blood to clot, the enzyme **thrombin** must be generated from the plasma precursor prothrombin. Thrombin then converts soluble fibrinogen into insoluble **fibrin** (see Blood lecture notes for clotting scheme). Generation of thrombin involves the sequential activation of a number of other plasma clotting factors, this process also being assisted by Ca^{++} and factors released by platelets and damaged tissues.

The time taken for blood to clot mainly reflects the time required for the generation of thrombin in this manner. If the plasma concentration of prothrombin or of some of the other factors is low (or if the factor is absent, or functionally inactive), clotting time will be prolonged. (Lack of fibrinogen is very rare and, therefore, unlikely to be a cause of prolonged clotting time).

Materials:

- Capillary tubes
- Sterile blood lancet
- Swabs

Procedure for Obtaining Blood from Finger:

- Swing your hand around until your fingers become engorged with blood.
- Cleanse the tip of the third or fourth finger with alcohol and give a quick, deliberate stab with a sterile lancet.

Procedure for Filling Tubes:

- Note the time the blood for examination first appears on the skin.
- Discard the first 2 drops and start filling with the third drop of **freely flowing blood.**
- Allow capillary action to fill 2 tubes simultaneously, by placing one end of each tube into the forming blood drop.
- Place the tubes on the table. At the end of 2 minutes **gently** break off approximately 1 cm length, every 30 secs from one tube, and look for a fibrin thread between the broken ends.

When this tube is finished, continue with the second tube. The time taken for a fibrin thread to form across the ends of the broken tube is the coagulation (clotting) time.

Record the time in your lab report.

II. Bleeding Time

Principle:

This test measures the time taken for blood vessel constriction and platelet plug formation to occur. No clot is allowed to form, so that the arrest of

bleeding depends **exclusively on blood vessel constriction and platelet action.**

Materials:
• Sterile blood lancet
• Circular piece of filter paper
• Alcohol swab

Procedure (Duke method):
The ear is used for this test. It should not be manipulated during the test but it should be warm.

• Clean the ear-lobe with alcohol
• Place the nail of your left thumb under the ear-lobe and make a quick stab with a lancet. **Note the time at that moment.**
• After half a minute, touch the top of the drop of blood formed - **not the skin** - with the edge of the filter paper and repeat this operation at half minute intervals.
The blood should **never** be wiped off, but must be completely removed by allowing it to flow onto the filter paper by capillary attraction. The end point, or **bleeding time**, is the first half minute when no blood is sucked up by the filter paper. **Record the time in your lab report.**

Two additional laboratory tests (not demonstrated here, but with which you should be familiar) are used commonly to evaluate coagulation disorders:

III. Prothrombin Time (PT)

Measurement: To a sample of blood which had been decalcified (by collecting it into a tube with oxalate or citrate ions), are added excess quantities of ionized calcium and some source of Tissue Factor (Thromboplastin, from e.g., homogenized brain tissue). Normally, the sample will clot within **10-15 seconds.**

PT measures the integrity of the **extrinsic system (Factor VII) as well as factors common to both systems (Factors X, V, Prothrombin, Fibrinogen)**. In the presence of all of these, PT will be normal. A deficiency in any of these will result in a prolongation of PT.

IV. Partial Thromboplastin Time (PTT)

Measurement: To a sample of decalcified plasma, are added kaolin (hydrated aluminum silicate), cephalin, and ionized calcium. Kaolin serves to activate the contact-dependent Factor XII, and cephalin substitutes for platelet phospholipids. Normally, the sample will clot in **35 seconds**. PTT measures the integrity of the **intrinsic system (Factors XII, XI, VIII, IX) and common clotting pathways.**
Note that sensitive assays for specific coagulation factors are now available.

PART C - BLOOD CELL INDICES

Among the most common hematological tests performed are those which determine the **red blood cell count**, the **hematocrit**, the **hemoglobin content**, (these 3 tests permit calculation of the Mean Corpuscular Volume (**MCV**) and Mean Corpuscular Hemoglobin Concentration (**MCHC**), the **total** and **differential White Blood Cell count**, and the **ABO/Rh Blood Group typing**.
Every one of these measurements may be carried out on a single drop of blood.

Assemble the materials for all the tests according to the list below.

I. RED BLOOD CELL COUNT

A convenient and inexpensive way to count blood cells is through the use of the hemocytometer (see p. 7-8). This instrument is a special microscope slide on which precise grids have been etched within a counting chamber designed to hold an exact volume of a diluted blood sample. The blood cell determination involves counting cells in several squares of the grid and obtaining an average number. That number is multiplied by a factor that compensates for the amount of dilution. The final result expresses **the number of red blood cells per cubic millimeter** of the original blood sample.

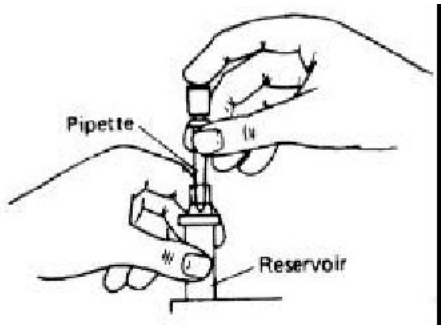
Materials for blood cell indices:

Obtaining Blood:
•small tube containing blood treated with an anticoagulant
WBC count and RBC count:
•WBC Unopette (smaller reservoir and tip marked “25”) •RBC Unopette (large reservoir and tip marked “10”)
•Hemocytometer
•Microscope
Hematocrit:
•glass capillaries
•"Seal-ease"
Hemoglobin concentration:
•Hemoglobinometer
•Hb. slide
•hemolysis applicator stick
White cell differential:
•prepared Difquik slide
•microscope
Blood typing:
•2 slides
•wooden or plastic stirring stick
•marker
•antisera: -Anti A, -Anti B, -Anti Rh
• Unknown commercial blood samples

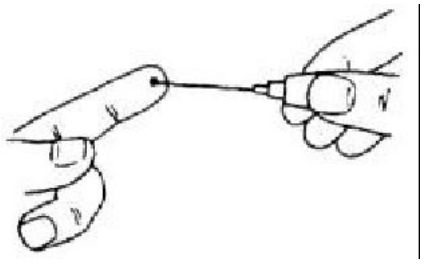
General Procedure

1- Have all the items in the list ready.
2- From blood supplied fill the hematocrit tube and seal it (see instructions on p. 9).
3- Fill Unopette capillary for red cell count (see instructions on p. 6 - 8) and have one partner start procedure for red blood cell count.
4- Fill Unopette capillary for white blood cell count (p. 6- 8).
5- Place a drop of blood on the slide used for determining hemoglobin concentration (see instructions on p. 8 and have another partner start procedure for this test.
6- The third partner starts identifying the white blood cell types on the prepared Difquik slide (p. 11).
7- Blood group determinations are also to be made (p. 11)
8- Once all the samples are processed, each partner should participate in:
• counting the red blood cells
• reading the hematocrit
• reading the hemoglobin level
• counting and identifying the white blood cells
• detecting agglutination (A,B,O,Rh identification)

Procedure:
a) Using the protective shield on the capillary pipette or tip of the Unopette, puncture the diaphragm of the reservoir (see diagram).

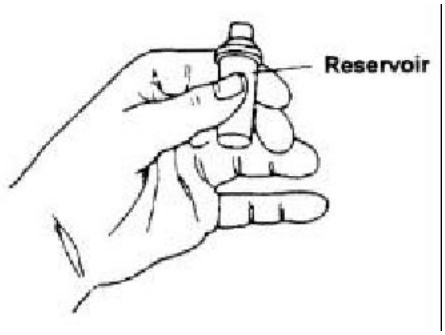


b) Remove the shield over the pipette and simply touch the tip of the pipette to the drop of blood. **The pipette will fill by capillary action**. Filling is complete and will stop automatically when blood reaches the end of the capillary bore in the neck of the pipette.

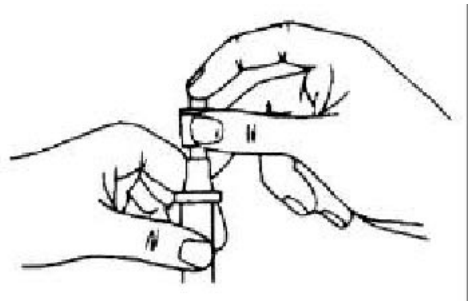


c) Carefully, **wipe excess blood** from outside of capillary pipette.

d) **Squeeze** reservoir slightly to force out some air. Maintain pressure on reservoir.

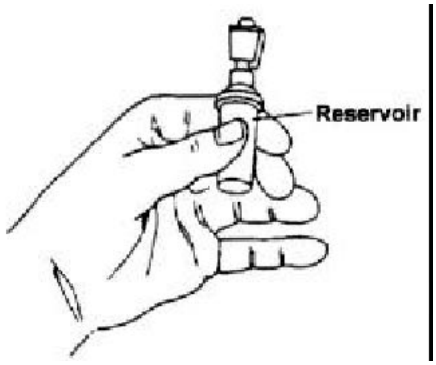


e) Cover opening of overflow chamber of pipette with index finger and seat pipette **securely** in reservoir neck.

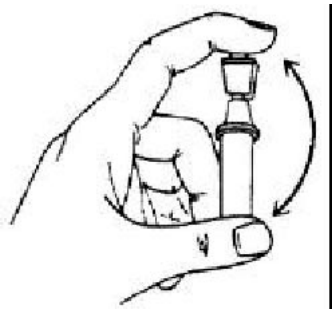


f) Release pressure on reservoir. Then remove finger from pipette opening.

g) Squeeze reservoir **gently** two or three times to rinse capillary bore, forcing diluent up into, **but not out of**, overflow chamber, releasing pressure each time to return mixture to reservoir.



h) Place index finger over upper opening and gently invert several times to thoroughly mix blood with diluent.

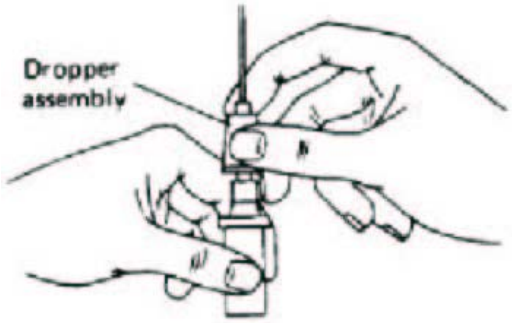


Charging Hemocytometer

Place the cover glass on the hemocytometer over the 2 counting

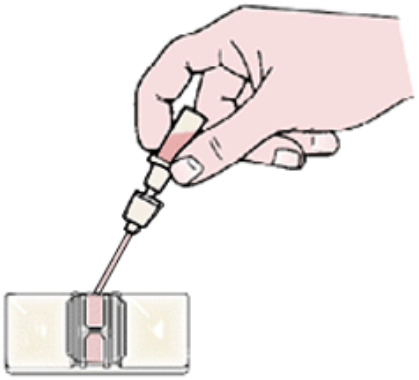
chambers and make sure that it is supported by both side bars.
Mix diluted blood thoroughly by inverting reservoir (see h above) to resuspend cells.

a) Convert to dropper assembly by withdrawing pipette from reservoir and reseat securely in reverse position.



b) To clean capillary bore, invert reservoir, gently squeeze sides and **discard first three or four drops.**

c) Carefully charge hemocytometer with diluted blood by gently squeezing sides of reservoir whilst applying tip of capillary to one edge of the chamber. **Capillarity will suck the cell suspension into the chamber**. Fill the other chamber in the same way with another drop from this same pipette. The drop should just cover the platform and **on no account should it be allowed to overflow into the moat** since an excess of cells will be suctioned off.

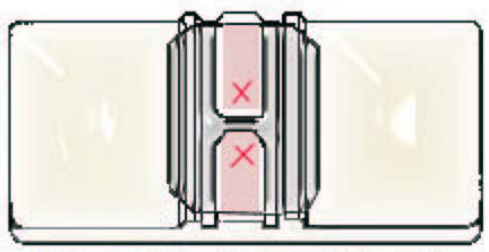


Enumeration

a) Locate the grid of the hemocytometer using the l0x objective. Focus on the large central square of the grid; change to 45x, refocus, adjust the light intensity so that the lines are clear and sharp, and the blood cells are clearly visible, and count the number of red blood cells in the areas indicated by the legend of the attached figure (See p. 9). To prevent counting the same cells twice, count cells that are touching the lines at the tops and left sides of the squares but do not include those touching the bottoms and right sides of the squares.

b) To calculate the number of red blood cells in a cubic millimeter of the blood sample, consider the following. The central grid of 25 squares is 1 mm^2 in area and 0.10 mm deep. The dilution factor is 1:200.

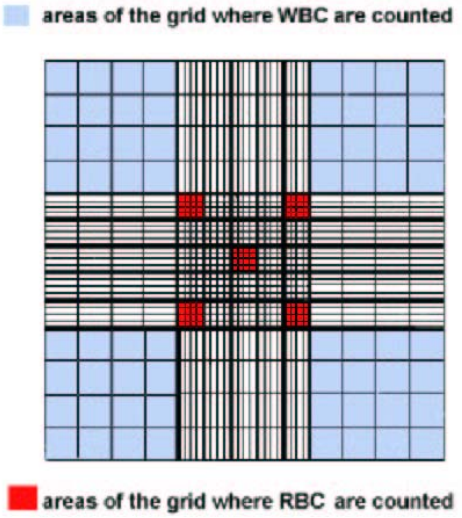
Hemocytometer



×areas where the grid is located

To convert the number of red blood cells that you counted in 5 squares

to the number of red blood cells/mm^3, you must multiply your count by 10,000 (the product of 5 x 10 x 200).



II. THE HEMATOCRIT

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**.

Procedure:

a) Touch one end of a heparinized capillary tube to the sample of blood provided. Allow the tube to fill to at least 3/4 capacity. (Air bubbles are not important, since they will disappear during centrifugation).

b) Holding a finger over the free end of the capillary tube, withdraw it from the sample of blood, and seal the blood-filled end with clay, by pushing it into the "seal-ease".

c) Place the sealed capillary tube in a numbered slot on the microcapillary centrifuge, with the plugged end of the tube facing outward and against the rubber gasket. **Remember your slot number**. When all the slots in the centrifuge have been filled, the **demonstrator** will turn the centrifuge on, allowing it to run for the appropriate duration. When the centrifuge has come to a full stop, remove your tube and determine the hematocrit, using the scale on the centrifuge.

III. HEMOGLOBIN CONTENT DETERMINATION

A common method for measuring the hemoglobin content of blood makes use of an instrument known as a hemoglobinometer, which compares the colour of light passing through a hemolyzed blood sample with a standard colour. The results of the test are expressed as **grams of hemoglobin per 100 ml of blood (g%).**

Procedure:

a) Examine the blood chamber; remove the metal clip to separate the 2 pieces of glass. Note that one of the pieces of glass has an "H"-shaped depression cut into it, while the other piece is flat on both sides.

b) Place a drop of blood on one side of the slide containing the "H"-shaped depression (see diagram).

c) Stir the blood with the end of a **hemolysis applicator which is**

coated with saponin (ruptures the cell membrane thereby releasing the hemoglobin) until the blood appears as a transparent red rather than cloudy liquid. This may take 30-45 seconds.

d) Position the flat piece of glass on top of the blood plate and slide both into the metal clip.

e) Slide the blood chamber into the slot on the side of the hemoglobinometer, ensuring that **it is in all the way.**

f) Holding the hemoglobinometer in your left hand so that your thumb is on the light switch on the bottom of the instrument, place the eyepiece to your eye and note the green area that is split in half.

g) Move the slide button on the side of the hemoglobinometer back and forth with the right index finger until the 2 halves of the green area look the same.

h) The index mark on the slide knob indicates the grams of hemoglobin per l00 ml of blood. (Read the percent hemoglobin on the 15.6 scale, which is taken as the standard for comparison).

Calculation of Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC)

Excessively low values of red blood cell count, hematocrit, or hemoglobin may be indicative of anemia (i.e. decreased oxygen carrying capacity of blood). There are many different causes of anemia (e.g. loss of blood through hemorrhage, bone marrow disease, iron deficiency, vitamin B_{12} or folic acid deficiency, etc.) and some of those are characterized by typically very small or very large red blood cells or reduced hemoglobin concentration in each cell. For example, iron deficiency anemia is described as **microcytic** and **hypochromic**, whereas vitamin B_{12} deficiency is **macrocytic** and **normochromic**.

Diagnosis of the type of anemia may be assisted by relating the measurements of **red blood cell count**, **hematocrit** and **hemoglobin** to derive the **mean** corpuscular volume **(MCV)**, and the mean corpuscular hemoglobin concentration (**MCHC**).

Procedure:
To calculate the MCV expressed in femtoliters (fl, 10-15L) - the following formula may be used

$$\mathrm{MCV} = \frac{\text{hematocrit (\%) x10}}{\text{RBC count (millions/mm}^3\text{ blood)}}$$

To calculate the MCHC - expressed as g of hemoglobin per 100 ml packed cells – the following formula may be used:

$$MCHC = \frac{\text{hemoglobin (g/100ml) x100}}{\text{hematocrit (\%)}}$$

IV. BLOOD GROUP TYPING

ABO System

The blood groups refer to the presence on human red blood cells of certain antigens, the blood group factors. One very important group of factors present on the red blood cells is the ABO system. The ABO group of a person depends on whether his red blood cells contain one, both, or neither of the 2 blood group antigens A and B. There are, therefore, 4 main ABO groups: A, B, AB and O. Antibodies (agglutinins) for the antigens A and B exist in the plasma and these are termed anti-A and anti-B. The corresponding antigen and antibody are never found in the same individual since, when mixed, they form antigen-antibody complexes, effectively agglutinating the blood.

In preparation to this lab, tabulate the antigens and antibodies found in individuals belonging to the ABO blood types:

Blood Group	Antigen	Antibodies
A		
B		
AB		
O		

Testing for ABO Group:

Procedure:

a) Label one end of a slide 'A' and the other end 'B'.
b) with the pipetman and a fresh tip, add 50 μl of anti-A test antiserum to the end marked 'A' and 50 μl of anti-B antiserum to the end marked 'B'. Use separate pipetman tips.

c) Add 50 μl of blood to each end of the slide and mix for 30 seconds, using separate wooden/plastic sticks. To avoid splattering the blood and antisera, do not press hard on the slide.

d) Read the results directly from the labels on the slides. The subject is blood group A if agglutination occurred on the 'A'-end of the slide; group B if agglutination occurred on the 'B'-end; group AB if agglutination occurred on both ends; and, group 0 if there was no agglutination.

Rh system

Rh antigens, named for the rhesus monkey in which they were first discovered, are also surface antigens expressed on red blood cells. There are a few Rh antigens (common one is called D). Red cells expressing the Rh antigens are called Rh positive. Red cells which do not express this surface antigen are Rh negative (about 15% of the human population is Rh negative).

Rh system becomes important when one considers the eventuality of Rh incompatibility between mother and fetus; in such a case, the antibody-mediated cytotoxicity mechanism involved threatens the well-being of the fetus.
During birth, a leakage of the baby's red blood cells often occurs into the mother's circulation. If the baby is Rh positive (inheriting the trait from its father) and the mother is Rh negative, these red cells will cause the mother to manufacture antibodies against the Rh antigen. The antibodies (IgG class) do not

cause problems for that first born, but can cross the placenta and attack the red cells of a subsequent Rh^+ fetus. The red cells are destroyed, leading to anemia and jaundice. The disease - erythroblastosis fetalis or hemolytic disease of the newborn- may result in fetal death.

Testing for Rh:

Procedure:

a) Add 50 μl of the anti-Rh test antiserum to another slide.

c) Mix well with another toothpick for 30 seconds.

Note that anti-Rh is structurally different from anti-A and anti-B, and is a weak agglutinator.

The test is positive if agglutination occurs within 2 minutes and negative if there is no agglutination within this time.

V. WHITE BLOOD CELLS: TOTAL AND DIFFERENTIAL COUNTS

Mammalian blood contains 5 different types of white blood cells (WBC) which can be distinguished by staining with dyes. The Difquik kit provides a rapid method for this purpose. In this test you will be (a) enumerating total leukocytes and (b) determining the percentages of each type of leukocyte present in the blood sample. The latter is termed a **differential WBC count**. Deviations from the normal values often indicate a diseased state, e.g., neutrophilia (high neutrophil counts) often signals localized infections such as appendicitis; eosinophilia may indicate allergic conditions or invasion by parasites such as *Trichinella*; lymphocytosis (high lymphocyte counts) may be seen in some viral infections. Neutropenia (a decrease in the count) occurs e.g. in typhoid fever, measles or infectious hepatitis, and eosinopenia may be produced by an elevated secretion of corticosteroids (in states of stress).

Total WBC Count

Proceed in exactly the same manner as outlined for the red cell count (p. 6), but use the **Unopette designed for WBC (smaller reservoir and capillary marked "25" in blue)** and count all of the cells in the 4 large squares coloured in lighter grey in the diagram (p. 9). Since the dilution factor is 20 and each of the 4 large squares counted has a volume of 0.1 mm^2, the number of WBC per mm_3 blood can be calculated by multiplying the total number of cells counted in 4 large squares by 50 - the product of

$$\frac{20}{4\times 0.1\text{mm}^3}$$

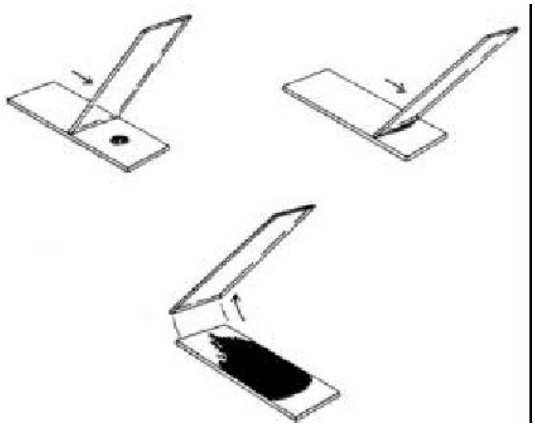
Differential WBC Count

Procedure (not performed in the lab) for the preparation of blood smears:
Put a drop of blood on a slide, 1" from one end, and spread with another slide in the manner illustrated in the figure. Note that the blood is dragged over the slide, not pushed. **Do not pull the slide over the smear a second time**. If you don't get an even smear the first time, repeat the process on a fresh clean slide. To get a smear that will

be the proper thickness, hold the spreading slide at an angle greater than 45°. When the smear is dry, stain it with Difquik.

Enumeration (to be done in the lab)

Place the prepared slide on the microscope and scan with the low power to find an area with a good distribution of cells. Place a drop of oil on the slide and examine it with the oil immersion objective (x100). First, practice identifying each cell type. Then count a total of 100 white blood cells and record the number of each type seen (e.g. 2 eosinophils, 25 lymphocytes, etc.). **Tabulate counts in the table on the next page.**



WBC Tabulation

Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes

Add the total number of each type of WBC and determine the grand total. Use this number to calculate the % of each type of WBC and enter these values in the table below. Multiply the % by the value you obtained for the total WBC count to calculate the number per mm^3 of each type of WBC, and add these values to the table below.

Leucocyte Types	Percent	Number of cells per mm^3(% x total WBC count)
Neutrophils		
Eosinophils		
Basophils		
Lymphocytes		
Monocytes		

Questions for Discussion
Blood

General questions
(To be considered in preparation for the Lab exercises)

1. Understand the purpose and potential clinical value of each blood test performed.

2. Understand the physiological principles that are being tested.

3. Make sure that you understand and are able to describe the steps of each test performed/demonstrated/described.

4. Make sure that you know the range of normal values for the results of each test performed/demonstrated/described. Be aware that some values are gender specific. Pay close attention to the units used.

5. Be able to distinguish between Bleeding Time, Clotting Time, Prothrombin Time, and Partial Thromboplastin Time.

6. Make sure that you understand the basis of antithrombic therapy (e.g., administration of aspirin), and for use of the different anticoagulant agents (e.g., Heparin vs. Coumadin)

7. Make sure that you are familiar with the broad principles of erythropoiesis (site, regulation, necessary raw materials)

8. Make sure that you are familiar with the distinguishable characteristics and functions of the different white blood cell types.

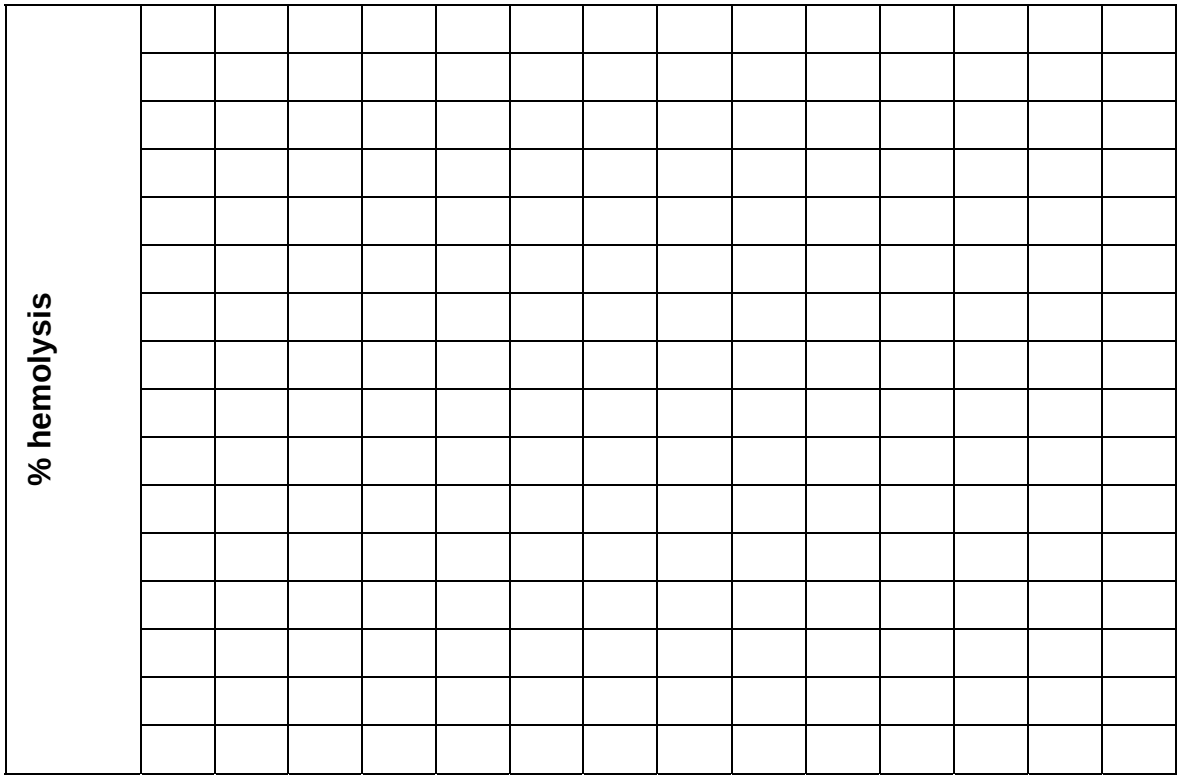
Results
Blood Lab

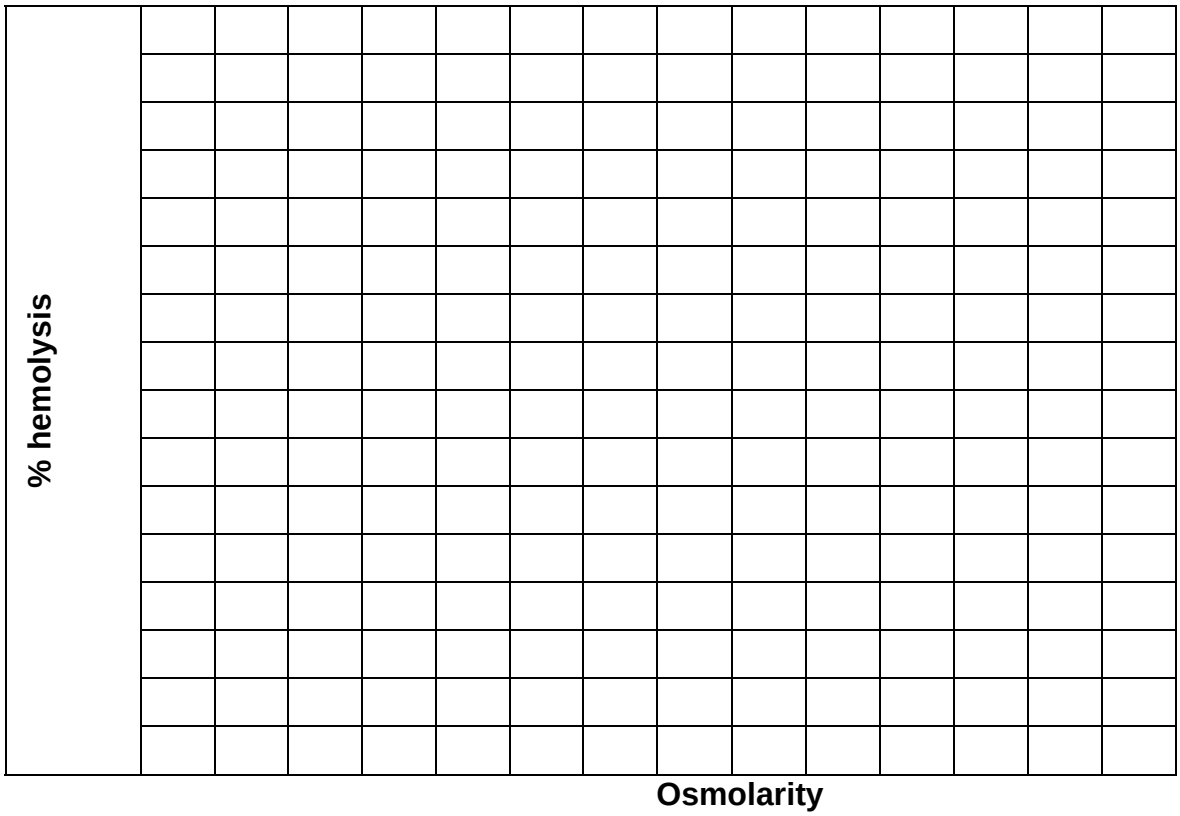
Name:
ID #:
Bench #:
Date:

Fill in the tables below with the results you obtained for the blood sample given.

Tube #	NaCl conc. g/100 ml	Molarity Mols/l	Osmolarity Osmols/l	Osmotic Pressure mm Hg	O.D. of sample	Percent Hemolysis % from curve
1						
2						
3						
4						
5						
6						

Measurement of Erythrocyte Fragility:





Fill in the tables below with the results you obtained for the blood sample given. Determine expected values for humans using your lecture notes or textbook.

	Video subject's	Expected human value
Clotting time (min)		
Bleeding time (min)		

	Blood sample value	**Expected human value (female)**	**Expected human value (male)**
RBC count ($10^6/\mu l$)			
WBC count (/μl)			
Hematocrit (%)			
Hemoglobin (%)			
MCV (fl)			
MCHC (gHb/100 ml)			
Blood type			
Neutrophil (%)			
Eosinophil (%)			
Basophil (%)			
Monocyte (%)			
Lymphocyte (%)			

a) Show sample calculations for Molarity, Osmolarity, and Osmotic Pressure of a given NaCl (MW = 58.5) concentration
b) Show sample calculations for the RBC and WBC Count
c) Show sample calculations for MCV and MCHC