Red Blood Cell tests (lab 6.1)

Background information and procedures for RBC tests

The background information and most of the RBC testing procedures for this lab are in lab 6.1. We will modify a few of the procedures, however. You should read lab 6.1, answer the lab report questions in the lab report section, and follow its experimental directions except for the changes explained below.

Precautions when working with blood samples.

A copy of the Hartnell College Blood Safety Procedures is reprinted on the last two pages of this handout. Please familiarize yourself with these procedures before starting any lab work involving blood. Some highlights of the safety procedure are listed below:

1) Only work with your own blood. You may not assist any other student in any way during today's laboratory.

2) Place an absorbent pad on your lab bench space before you begin any bloodwork. All of your activities must be performed over the pad.

3) No materials go into the regular trash.

Put all hard or sharp wastes (Lancets, toothpicks, capillary tubes etc.) in the sharps container.

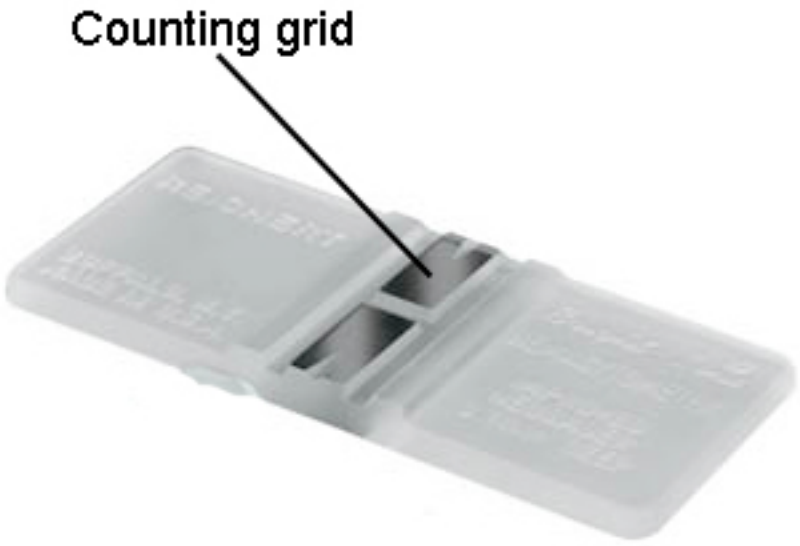
Put all soft wastes (absorbent pads, paper towels, alcohol wipes, etc.) in the biohazard bucket.

4) After the experiment, clean the hemocytometer glass slide and the cover slip with water. Dry them and put them back in their cases.

5) After the experiment, clean the surface of the lab bench (and any other locations in the room where you may have left blood) with a disinfecting agent.

a) Procedure for Red Blood Cell Count (procedure A)

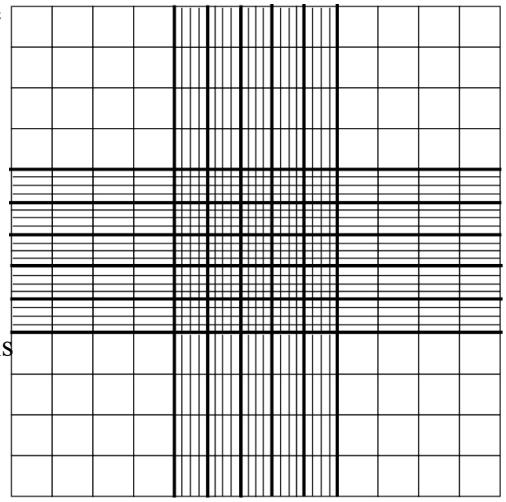
1) Obtain a plastic box containing a hemocytometer (a special microscope slide with a counting grid for RBCs) and a cover slip. Use the hemocytometer number that matches your microscope number.



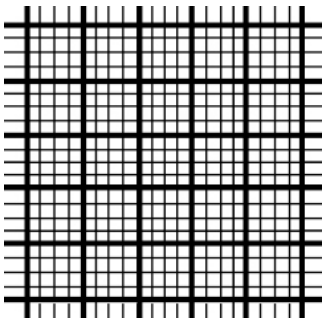
2) On the front desk is a test tube containing synthetic blood diluted 1:200. The instructor will place one drop on the counting grid of the hemocytometer. Then you should place the cover slip on top of the blood drop.

3) Put the hemocytometer on your microscope stage. The counting grid is difficult to focus on unless you first adjust the light properly. Turn the rheostat (the bulb voltage) to maximum but adjust the condenser iris diaphragm to as dim as possible.

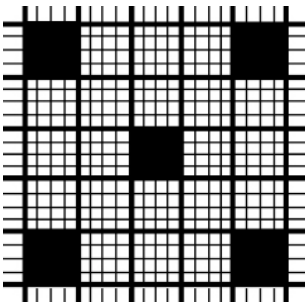
4) Start with the 4X objective lens. Locate the grid by turning the course focus knob up and down. It should look like the one the right. If you are not able to see the grid, ask your instructor for assistance.



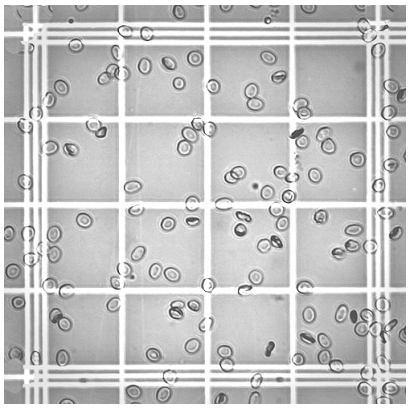
5) Once you have the grid in focus with the 4X lens, center the counting grid in your field of view. Now change to the 10X objective lens and refocus. The central grid image should look like the one shown below:



Notice the central grid is a large square divided into 25 smaller squares. You are going to count RBCs in 5 of the 25 squares. The 5 squares you will count are the shaded ones shown in the figure to the right.



6) Once you have the central grid in focus with the 10X lens, center the first counting square (the one in the upper left) in the field of view. Now change to the 40X objective lens and refocus.



7) Using the 40X lens, the first counting square should appear similar to the one shown on the right. The counting square is the square with triple lines, not the single lined squares within it.

8) Count all the RBCs that you see in this counting square. Include in your count any RBCs that are touching the top or left edges of the square. Don't include any RBCs that are touching the bottom or right edges. It may be helpful to use a clicker-counter from your physiology basket.

9) When you have finished counting all the RBCs in counting square 1, move on and count counting squares 2. 3, 4, and 5.

Record your counts here: Sqr1 = _____
Sqr2 = _____
Sqr3 = _____
Sqr4 = _____
Sqr5 = _____

10) Add together all the RBCs you counted in the 5 squares, then multiply this total by 10,000. The value you get is the RBC count for the blood sample. Enter the RBC count in the data table in this handout.

11) Wash the slide and the cover slip with water. Dry them gently and put them back in the plastic container. The cover slip is very delicate.

b) Procedure for Hematocrit test (procedure B)

Before starting this procedure, obtain a puppy pad, a capillary tube, a sterile lancet, a piece of Talquist paper, an alcohol swab, and a paper towel. Place the puppy pad and all the items on your desk top. Do all of your blood work over the puppy pad and keep all of your items on the pad.

1) Puncture your finger using a sterile lancet (follow the lancet instructions given by your instructor).

2) Fill the capillary tube with your blood. Also, spot a drop of blood on the Tallquist paper.

3) Seal the end of the capillary tube with a clay plug. Push your tube into the clay box gently or the capillary tube may break.

4) Load your capillary tube into a slot in the centrifuge.
   (a) Be sure to note your slot number.
   (b) Be sure the yellow plug is to the outside of the rotor.
   (c) Be sure another capillary tube is exactly 180 degrees opposite yours for balance.
   (d) Be sure the screw-on safety lid is on the rotor before centrifuging.

5) Centrifuge the capillary tubes for 3 minutes at full speed.

6) Obtain a metric ruler from your physiology basket. Measure the volume of the RBCs (the lower red layer) and the total blood volume (the lower RBC layer and the upper clear plasma layer).

7) Calculate the hematocrit: This is the % of the total blood volume that is RBCs. Record the hematocrit in the data table in this handout.

c) Procedure for Hemoglobin Concentration test (procedure C)

1) You should already have spotted a drop of blood on a piece of Tallquist paper. Hold the blood spot behind the color scale card. Match the color of the blood spot with the square on the card that is closest in color.

2) Record the hemoglobin concentration (the grams per 100 ml value, not the % value) in the data table in this handout.

3) Clean up: Scrub the color scale card with disinfectant. Put your lancet and the capillary tube in the sharps waste. Put the puppy pad, the Tallquist paper, and any other non-sharp wastes in the soft biohazard wastes container.

Results table:

A) RBC count: _____

Was this within the normal RBC count range? _____

B) Hematocrit: _____

Was this within the normal hematocrit range? _____

C) Hemoglobin concentration: _____

Was this within the normal concentration range? _____

Safety procedures for working with human blood in the laboratory

All faculty and students will use the following procedures for safe handling and disposal of human blood samples in the laboratory.

Instructors must inform students of the policies outlined below and discuss the potential dangers of exposure to blood-borne pathogens. Instructors must enforce the policies by whatever means are necessary to ensure compliance.

1) Each student will work only with their own blood sample. A student may not assist another student in puncturing skin, drawing blood, working with blood samples, cleaning work area, disposing of materials, or any other activity where blood is handled. Students are not permitted to enter another student's work area when blood work is being done.

a) The instructor will not provide any bloodwork-related equipment or materials to students who are present in the laboratory but not working with blood.

2) Work areas (such as laboratory bench tops) that will be used for blood work will be covered with a disposable absorbent material for the duration of the work. Students will work with their blood only in their own work area and are responsible for cleaning that work area, lancet pens, and other equipment contaminated with only their own blood, and for disposal of their own blood work supplies.

a) The instructor is responsible for cleaning blood on shared areas and shared equipment (such as the centrifuge, the hematocrit reader, and the capillary tube sealer) and for cleaning any blood of unknown origin.

b) Any blood on shared areas, shared equipment, or outside of the student work area will be cleaned immediately.

3) All disposable materials that have contacted blood or could reasonably be assumed to have contacted blood are to be considered biohazardous materials and must be disposed of in special containers as outlined below:

a) Soft materials (such as absorbent pads, paper towels, dressings, gloves, gauze, and cotton balls) must be placed in a labeled biohazard bag and autoclaved. The bag must be taped whenever transported.

b) Sharp materials (such as lancets, needles, toothpicks, capillary tubes and other disposable glass) must be placed in a labeled plastic biohazard container immediately after use and autoclaved. The container lid must be closed whenever transported.

c) Broken, contaminated glassware must not be handled directly with hands, but must be cleaned up by mechanical devices such as brush and dustpan or forceps.

4) Surfaces and non-disposable equipment that have contacted blood or could reasonably be assumed to have contacted blood are to be cleaned and decontaminated using a solution capable of sterilizing/disinfecting the surface/equipment (such as hydrogen peroxide, staphene, or 1:10 diluted bleach solution).