Colby College BIOLOGY 225 Spring 2013
Prof. Hannum

Lab 1
Blood cells

Part A. Identifying human leukocytes using Wright stain

We will use commercially prepared smears of human blood for this exercise. Each pair of lab partners will stain one slide, then take an inventory of the leukocytes present.

Staining procedure - use gloves

1. Place 9 drops of Wright stain onto the blood smear. Let it stand for 3 minutes.
2. Add 9 drops of buffer. Mix the stain and buffer by gently blowing on the liquid.
3. After 1.5 minutes, drain the buffer off the end of the slide.
4. Add 9 more drops of buffer to the slide. Let stand for 8 minutes.
5. Flush the slide with distilled water.
6. Drain as much water off the slide as possible (wipe the back of the slide with a paper towel). Wave the slide around vigorously to dry the stained blood smear.

Examining the smears

Observe the stained smears using the microscopes in the lab bench cabinets. Begin by using the lowest power objective (4x), then view the slides with the higher power objectives.

Scan the smear systematically, identifying the first 100 leukocytes you see (each person in a lab pair should do 50). Tally the numbers as you go in your lab notebook. Record the final counts in your lab notebook, and then on the lab spreadsheet.

Part B. Counting Cells

Using a hemacytometer to count cells

A hemacytometer has two counting chambers, each with a tiny 9mm^2 grid etched onto it. When a drop of cell suspension is added to the loading groove, the liquid spreads out under a special, weighted cover slip. The protocol for counting is on the following page. First, let's see what those counts mean…

When loaded, the distance between the grid and cover slip is 0.1mm. Therefore, when you count the number of cells in a 1mm^2 grid (16 "squares") you are actually counting the number of cells in 0.1mm^3 = 0.1 microliter (μl).

Once you've counted the cells on a hemacytometer you must then **convert** number of cells per 0.1 μl to number of cells per ml of your original suspension:

cells per 0.1 μl = # cells counted x dilution factor, thus

cells per 1 ml = # cells counted x dilution factor x 10000, or

 # cells per ml = average # cells counted x dilution factor x 10^4

For example

If your sample is undiluted (dilution factor = 1) and you count an average of 56 cells per 16-square block, then

cells/ml = 56 x 1 x 10^4

= 5.6 x 10^5 cells/ml

if you diluted your sample 1:10 before counting (dilution factor =10) and you counted an average of 25 cells, then the # of cells/ml = 25 x 10 x 10^4

= 2.5 x 10^6 cells/ml

Practice

If you dilute a suspension of cells 1:20, then count an average of 15 cells per 16-square grid, what is the concentration of cells in your original cell suspension? Express your answer in cells/ml.

Part B, continued

Cell counting protocol

Today we will use a cultured cell line to practice counting.

1. Place **90 µl cells** and **10 µl trypan blue** in a microcentrifuge tube and pipet to mix.

2. Draw cell mix up into a pipette tip or pasteur pipette. Release just enough liquid so that a drop hangs from the end of your pipette tip. Touch the hanging drop to the loading groove of the hemacytometer, right where it meets the cover slip.

- Capillary action will draw your sample under the cover slip.
- Do not inject or force liquid under the cover slip or the volume will be too high, making your count inaccurate.
- If you overload the hemacytometer, just wipe it off with a Kimwipe and start again.

3. Place the hemacytometer on an inverted scope with the general area of the grid over the objective.

4. Observe with 4x objective first (remember, this is a total of 40x magnification). Once you've found the grid and get it into focus, switch up to the 10x objective. The cells may not all be in focus yet, as it takes a minute for them to settle to the bottom of the counting chamber.

5. Count live (clear) and dead (blue) cells in four 16-square grids under 100 or 200x magnification. Record the counts and calculate the average count per 16-square grid in your notebook.

Questions

Calculate the number of cells/ml in your suspension.

Calculate the percentage of viable cells (# live cells/#total cells) x 100.

Notes for instructors:

Part A
I use the "Wright Readi-Stain kit" from Carolina Biological (#700380) for the blood smear staining.

During this lab we talk about the format of figures in scientific papers. As an assignment, each student generates a bar graph depicting the mean number of leukocytes of each type identified ± SE, plus a brief figure legend.

Part B
Cultured cells are the easiest to use here, since they're fairly large and uniform, but fresh mouse spleen cells (with RBCs lysed) can be used too.

We only have 3 hemacytometers, so groups take turns counting cells while other groups are staining the blood smears.