Glucose determination (lab 2.1A)

A) Background information on carbohydrates and spectrophotometry

The background information for understanding this lab is found in in lab 2.1A in the lab manual.

Note that today's experiment is a *simulated* blood glucose test. No real blood is used. You do not need to wear gloves and you may dispose of all materials down the sink.

B) Preparing the glucose solutions

1) Obtain the following materials from your physiology basket and from the tables in the classroom.

a) 8 glass test tubes in a test tube rack.
b) Wax pencil to number the test tubes.
c) 100 ml graduated cylinder.
d) 5 ml serological pipette with a pipette device
e) p200 micropipette with a disposable tip
f) Laptop computer with power cord
g) SpectroVis desktop spectrophotometer
h) A square-sided plastic cuvette

2) Number the test tubes 1 – 8. Use the micropipette to transfer 50 ul of the following solutions into the eight test tubes. Remember, use the micropipette's first stop to draw a sample into the micropipette, and use the second stop to deliver the sample into your test tube.

Test tube	50 ul of Glucose solution (g/l)
1	0
2	0.25
3	0.5
4	0.75
5	1.0
6	1.25
7	Mr. A blood sample
8	Mr. B blood sample

3) From the front desk, your instructor will put 30 mL of Biuret reagent into your graduated cylinder. Back at your desk, using the serological pipette to transfer 3 mL of Biuret reagent you're your graduated cylinder into each of your eight test tubes.

C) Finding the absorbance of the glucose solutions

1) Plug the power cord into the computer and turn on the laptop computer. The password is student. Plug the spectrophotometer to the computer using the USB cable.

2) Open the Logger Pro program on the computer desktop. A rainbow spectrum should appear in the center window.

3) Add the contents of tube 1 (the zero glucose tube) into the cuvette (the square-sided test tube).

- Note that the cuvette has two clear sides and two rough sides.

- To avoid getting any skin oil contamination on the clear sides, hold the cuvette only on its rough sides and wipe the clear sides of the cuvette with a tissue before putting the cuvette into the spectrophotometer.

Place the cuvette into the spectrophotometer. One clear side of the cuvette should face the white arrow icon on the spectrophotometer and the other clear side of the cuvette should face white light bulb icon on the spectrophotometer.

4) On menu at the top of the screen, navigate through the following windows: Experiment>Calibrate>Spectrophotometer. The calibration window may delay 90 seconds to warm up the lamp. When the 90 second warm up is done, click Finish Calibration. Then click okay. **Note that solution 1 is the ONLY solution you should calibrate with.** In other words, do **NOT** do any more calibrations today.

5) At the top of the screen, click the green collect button. (If a small window pops up, select Erase and Continue). A table on the left of the screen will show the absorbance of the solution at many different wavelengths of light. Lastly, click the red stop button at the top of the screen.

6) Scroll down the table on the left to find the solution's absorbance at 550 nm wavelength.

7) Record the solution's absorbance in the data table in this handout.

8) Dump out the solution in the cuvette back into test tube where it came from. Pour the next solution into the cuvette, wipe the clear sides of the cuvette with a tissue, then place the cuvette back into the spectrophotometer, with the cuvette's clear sides again facing the white arrow and bulb icons.

9) Repeat steps 5 - 8 with each solution, but remember that you **do not recalibrate.** In other words, the calibration (step 4) was only done with tube 1.

10) When done, show your instructor your results. When your instructor has approved your results, shut down your computer and put all electronic equipment back where you obtained it.

Do not yet dispose of your solutions yet. Only dispose of them after your instructor has approved your graph of the data (in section D, below).

When your instructor has approved of your graph, then clean up. To clean up, dispose of the Biuret solutions in your glass test tubes in the special Biuret waste container in the fume hood. Then wash all glassware thoroughly. The final wash should be with de-ionized water. Be sure to scrub the wax pencil numbers off of your test tubes. Also wash out the plastic cuvette with deionized water and put the cuvette back where you found it.

D) Graphing the six standards

The six solutions of known glucose concentration are called the "standards" of today's experiment, because they have known values: (0, 0.5, 0.75, 1.0, and 1.25 g/l). The blood samples from Mr. A and Mr. B are called the "unknowns" of the experiment because their glucose concentration is not known to you.

In this section you will graph the absorbance vs. the glucose concentration for the six standards. It may be helpful to review the math handout section on graphing. Do not graph the two unknowns.

1) On the graph paper at the end of this handout, label each axis. Remember that a fully labeled axis has a phrase describing what is being shown and (in parenthesis) the units that are being used.

2) Number each axis correctly. Recall these rules for proper numbering:
   a) The numbering starts at zero

   b) Place your highest data value at (or near) the end of the axis.

3) When the both axis have been labeled and numbered correctly, show your labeled axes to your instructor.

4) Plot the six standards on the graph. **Use the unit conversion factor method to place each data point at its exact correct location on the graph.** Do not guess or estimate.

5) Draw the best fit straight line through the data points. Recall that the best fit straight line is one continuous straight line that (a) goes through 0,0, and (b) has equal numbers of data points above and below the line.

6) Show your instructor your graphed data points when done.

E) Determining Mr. A's and Mr. B's blood glucose concentration

A graph of experimental standards (such as the one you just completed) is called a "Standard Curve". In this section you will use the standard curve to find the blood glucose concentrations of Mr. A and Mr. B from their absorbance values.

1) Find the exact line on the absorbance axis of the graph that matches Mr. A's absorbance. **Use the absorbance unit conversion factor to find the exact line of Mr A's absorbance.** Do not just estimate the line's location.

2) Once you have found the correct line for Mr. A's absorbance, draw a horizontal line from there straight across to the best-fit straight line.

3) Where the horizontal line intersects the best fit line, draw a vertical line that goes straight down to the glucose concentration axis.

4) Where the vertical line intersects the glucose concentration axis is Mr. A's blood glucose concentration.

Use the glucose unit conversion factor to convert the line number on the glucose axis into a glucose concentration value for Mr. A.

5) Repeat steps (1) and (4) for Mr. B. When you are done, show your instructor you values for Mr. A and Mr. B.

F) Converting Mr. A's and Mr. B's blood glucose concentrations into milligrams per 100 mL.

The blood sugar concentrations for Mr. A and Mr. B that you determined from your standard curve are in units of grams sugar per liter of blood (g/L). Blood glucose levels, however, are usually reported in milligrams of glucose per 100 mL of blood (mg/100 mL).

Use the unit conversion factor method to convert the g/L concentrations into mg/100 mL. Note that 100 mL is the same as 1 deciliter. It is easiest to convert from g/L into mg/dL and then simply re-write the dL on the bottom as "100 mL".

On the whiteboard your instructor will write an example conversion from g/L to mg/100 mL

When done, show your instructor you values for Mr. A and Mr. B. in mg/100 ml.

Results table:

Test tube	Glucose concentration (g/l)	Absorbance:
1	0	_____
2	0.25	_____
3	0.5	_____
4	0.75	_____
5	1.0	_____
6	1.25	_____
7	Mr. A blood sample	_____
8	Mr. B blood sample	_____

Mr. A blood glucose = _____ g/l.
Mr. B blood glucose = _____ g/l.

Mr. A blood glucose = _____ mg/100 ml.
Mr. B blood glucose = _____ mg/100 ml.