Student Guide

Purpose: The purpose of the lab is to measure the glucose concentration of several synthetic blood and urine samples to determine which patients have diabetes.

Introduction to the activity:
Do you know someone with diabetes? It is becoming a major health issue in the U.S. According to the 2011 National Diabetes Fact Sheet, 8.3% of the population (25.8 million) have diabetes. Another 79 million people are in pre-diabetes with 1 in 4 children and adolescences having the disease.

What is this disease? *Diabetes mellitus* is a metabolic disorder that is characterized by the lack of insulin production, which results in elevated blood glucose. Insulin is a protein that allows cells to take up glucose from the blood for metabolism into energy (ATP) for bodily functions. To manage diabetes, patients require multiple insulin injections daily and rely on glucose monitors to measure their blood glucose for accurate insulin dosage [1].

Blood glucose monitors are sensors that use chemistry and electricity to measure the concentration of glucose in the blood. These fall under the growing number of devices called "lab-on-a chip." Many people are familiar with home pregnancy tests and instant strep tests. The use of nanotechnology has allowed monitors to become smaller, more sensitive, and measure smaller volumes of blood or other fluids. Blood glucose monitors operate using basic chemistry concepts. Blood is placed on the testing strip and the glucose in the sample reacts with glucose oxidase, an enzyme, to produce gluconic acid. The gluconic acid reacts with ferricyanide, a chemical on the testing strip, to produce ferrocyanide. The ferrocyanide reacts with the electricity generated by the meter to produce a reading of the blood glucose concentration of the patient [2].

Nanotechnology researchers have also been able to develop a detector that allows diabetics to monitor glucose levels in their blood using single-walled carbon nanotubes. The carbon nanotubes are formed from millions of carbon atoms and fluoresce (glow) at particular wavelengths in the electromagnetic spectrum in the presence of blood glucose. Scientist have inserted the detectors into human tissue samples and illuminated it with an infrared laser, which has allowed them to relate the glucose concentration to the strength of the fluorescence. Transducers were used to convert the florescence into an electrical signal that can be displayed on a digital screen as the glucose concentration.

You may want to explore the *Nanooze* issue on nanomedicine which has an article on nano tattoos and blood glucose. (http://www.nanooze.org/english/pdfs/nanoozeissue_08.pdf)

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Materials:

- Blood Glucose Monitor kits (5, recommend NovaMax meter)
- Blood glucose standard (5, included with monitor kit)
- 2 mL of simulated blood samples (3 samples/group, See protocol below)
- 2 mL simulated urine samples (3 samples/group, See protocol below)
- Microscope Slides
- Blood glucose test Strips
- Urine glucose test strips
- Disposable gloves
- Paper Towels
- Glucose tablets or glucose powder
- Yellow food coloring
- Red food coloring
- Disposable transfer pipets
- Beakers
- Graduated cylinder or pipets
- Stirring rods or magnetic stirrers
- 2 mL centrifuge tubes for blood samples

Follow the instructors of your teacher for these activities

Activity I: Blood Glucose Monitor and Urine Analysis

Test blood glucose monitor:

1. Remove the plastic check strip that comes with your kit.
2. Insert the check strip into the test port of the monitor with the dimple facing up.
3. The monitor should turn on and should prompt for blood collection.
4. If the monitor does not work, take the strip out and rotate it 180 degrees.
5. If the instrument still does not work, call your teacher**.**

I. Test Monitor for accuracy

1. Insert the testing strip into the meter (make sure you are wearing gloves)
2. Using the blood glucose standard that is included with your monitor, take a measurement of the substance by placing a small drop on the testing strip
3. Wait 5 seconds for the measurement to display. Record the value.
4. Repeat two more times
5. Calculate the average blood glucose
6. Compare your value to the concentration shown on the bottle. If your value is in the range of the manufacturer's value, then proceed to Part II.

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7. If your value is outside of the manufacturer specification, test a different vial of the standard and contact your teacher.

	Blood glucose measurements (mg/dl)			
Monitor Value	1	2	3	Ave
Kit blood				

II. Measuring blood and urine glucose concentration

Blood testing

a) One at time, place a sample of the patient's blood on the labeled microscope slide using the transfer pipet. **Be careful not to mix the blood samples and wear gloves at all times.**

b) Touch the testing strip to the blood sample and remove when the meter beeps and is calculating the glucose concentration

c) Record the value of the glucose concentration for the patient and remove the testing strip. Discard each strip after use.

d) Install a new strip and repeat the experiment two more times for each blood sample.

Urine testing

a) Dip the testing strip into the urine sample. Make sure to wear gloves at all times.

b) Determine the glucose concentration by matching the testing strip to the corresponding color on the strip bottle. Discard the used strip.

c) Test each sample three times using a new strip for each trial and record the results in the appropriate table.

Activity:

You are a doctor who is administering physicals for three patients. You will use nanotechnology to determine if your patients have normal, high, or low glucose values. Patients with low glucose values are hypoglycemic and will be prescribed a high glucose diet to maintain normal glucose levels. Those who have high blood or urine glucose values may be diabetic and may require future insulin therapy. Test the patient's fasting and post-meal samples to determine the status of their blood and urine glucose levels.

I. Pre-meal values

When the patients came to the doctor's office, their blood and urine was collected to measure their fasting (pre-meal) glucose levels.

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1. Determine the pre-meal blood and urine glucose values of the three patients.

Pre-meal Sample	Blood glucose measurements (mg/dl)			
Patient	1	2	3	Avg.
1				
2				
3				
	Urine glucose measurements (mg/dL)			
Patient	1	2	3	Avg.
1				
2				
3				

2. Calculate the average value for each patient.

II. Post-meal values

The patients were given a carbohydrate-rich meal of rice, chicken, orange juice, and ice cream sundaes. Blood samples and urine were collected two hours after the meal.

1. Determine the post-meal blood and urine glucose values of the three patients.

Post-meal Sample	Blood glucose measurements (mg/dl)			
Patient	1	2	3	Average
1				
2				
3				
	Urine glucose measurements (mg/dL)			
Patient	1	2	3	Average
1				
2				
3				

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2. Calculate the average value for each patient and fill in the chart. How do the values compare to each other?

Analysis & Conclusion

1. Graph the pre- and post-meal data for the blood and urine glucose concentration for each patient. How do the values for the patients change after having a meal?

2. Which patient was diabetic, normal, and hypoglycemic?

3. Why was it important to conduct a quality control test on the blood glucose monitor before you started taking data on the blood samples?

4. How did the blood glucose value change for the normal person after having a meal? Why?

5. Why is insulin important for the body?

Activity II: Modeling the glucose concentration in the body due to the action of insulin

In this activity you will investigate the conservation of mass of glucose to further your understanding of the role of insulin on glucose metabolism.

The conservation of mass law states that mass is not gained or destroyed during chemical reactions. For example in the equation below, when oxygen and hydrogen are combined to form water, the number of oxygen and hydrogen atoms in the products must equal the reactants.

$$O_2 + 2H_2 \longrightarrow 2H_2O$$

Figure 1: Illustrating the conservation of mass in the formation of water. The number of oxygen and hydrogen atoms in the reactants and products are in the same.

In this activity you will build a model to illustrate the conservation in the presence (non-diabetes) and absence of insulin (diabetes). In order to build the model, you must determine the following:

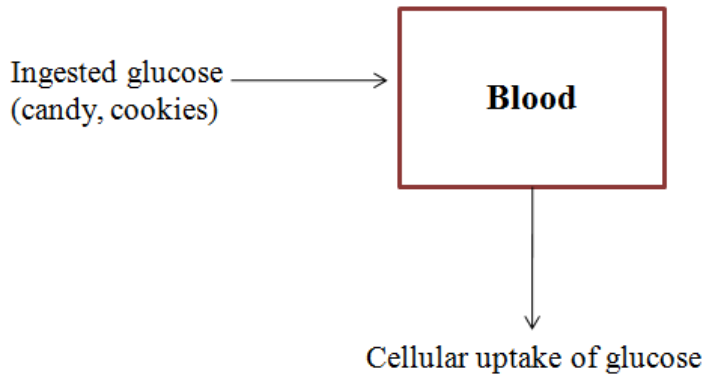
I. Create a model to determine the accumulation of glucose in the blood for the following scenarios. Make sure you list all assumptions. Use the conservation of mass equation below to help create the model.

In – out= accumulation- consumption

In (I)=glucose coming into the blood
Out (O)=glucose leaving the blood
Accumulation (A)=amount of glucose building up in the blood
Consumption (C)-amount of glucose being used for chemical reactions
We will assume that C is 0 for the model.

Revised mass balance equation: I-O=A

A. To get started the model for a non-diabetic person is below



Model Equation

I – O= A

Based on previous results you will find that accumulation of glucose in the blood is zero since insulin is present, so the mass balance for the non-diabetic patient is below.

I = O

For example, if 45 g of sugar is ingested, then we can roughly estimate that all 45 g will be taken up by the cells.

B. Create a model for someone who has untreated diabetes. List all assumptions. What is the conservation of mass equation for this person? Hint: The body does not like to accumulate large amounts of sugar in the blood.

Model picture

Model Equation

C. Create a model for someone who has untreated diabetes and non-functioning kidneys. List all assumptions. What is the conservation of mass equation for this person?

Model picture

Model Equation

Questions

1. What is the source of the glucose input for the model?
2. What are the ways in which glucose can leave the blood?
3. Why do people with untreated diabetes drink a lot of water and urinate frequently?
4. How does lack of insulin affect the untreated diabetes model?