The Breakdown On Glucose

(Enzymatic Oxidation of Glucose Via Glucose Oxidase and the Use of Glucometers in the Measurement of Blood Glucose Concentrations)

Katelyn Nguyen with contributions from Sara Ayele, Brian Britton, Stacy Chen, Lauren Flynt, Alida Huynh, Jessica Lam, Rachel (Doo) Lim and Kristen Nuckles

Virginia Commonwealth University School of Pharmacy
410 North 12th Street
Richmond, VA 23298-0581

ABSTRACT

Diabetes is a rising epidemic in the U.S. affecting millions of people. The use of glucometers has become a beneficial aid to these patients to help them monitor their condition on a continuous basis. However, there's a plethora of commercially available glucometers in the market that have similar functions, but also slight differences between them depending on the testing conditions. Thus, it's important to know how these instruments work and what these differences are in order to make the proper recommendations to patients. Two separate experiments are done to examine how glucose oxidase functions within a given glucometer and how certain experimental and instrumental variations may affect the glucose readings at the end. The results of the experiments indicate that temperature could affect the final glucose reading so it's one factor that needs to be taken into consideration when doing the tests. Additionally, the improper use of the test strips and not following the manufacturers' recommendations can drastically affect the glucose readings. Many factors (environmental, experimental, individual, etc.) may affect the glucose measurements when working with these glucometers so special attention need to given to reduce the effects of these conditions. Proper counseling is also important to ensure that patients understand how to perform these tests at home and be aware of certain external factors that may affect the results.

INTRODUCTION

Glucose is an essential component of the body's metabolism because it is a source of energy for the body's many functioning systems such as muscle cells and brain tissues. Although glucose is a fundamental element in the body, too much of it can lead to serious health problems such as diabetes mellitus, a group of diseases that affect how the body uses blood glucose.

Diabetes mellitus is a health condition that affects more than 20 million Americans with over 40 million Americans having pre-diabetes (early Type 2 diabetes) (2). The lifelong (chronic) disease is characterized by unusually high concentrations of glucose in the blood due to abnormal metabolism of sugars in the body (5). Diabetes is often caused by having too little insulin or resistance to the existing insulin in the body. Insulin is a hormone produced by the pancreas to control blood sugars by moving glucose from the bloodstream into fat, muscle and liver cells where it is used as fuel for the body (2). Diabetic patients have trouble moving sugars into their cells to be stored as energy because their pancreas may not be producing enough insulin or their cells do not respond to the insulin produced leading to insulin resistance (2).

There are currently three major types of diabetes: Type 1 Diabetes, Type 2 Diabetes and Gestationa Diabetes. Type 1 diabetes, also known as juvenile-onset diabetes, occurs mostly in children, but can affect any age group (1). This particular type requires daily injections of insulin because the body makes little or no insulin of its own. Type 2 diabetes results from insulin resistance (body rejecting the insulin the pancreas produces) and occurs most often in adulthood affecting mostly obese patients. Gestational diabetes is high blood sugar that develops during pregnancy in women who normally don’t have diabetes (2).

Diabetes is becoming more prevalent in today’s society especially with the increased obesity rates so patient monitoring is important to ensure that proper measurements and treatments are taken to protect the patients. Various blood tests are used for screening and to confirm the diagnosis for diabetes especially for patients with blood levels higher than 200 mg/dL (2). Three types of blood tests can be done to measure blood glucose levels each with its own standards of measurement and target ranges to confirm the diagnosis for diabetes. They include the fasting blood glucose test, Hemoglobin A1C test, and the oral glucose tolerance test. Patients who are at high risk for diabetes should take precaution and get screened annually especially if they are overweight (BMI greater than 25 for adults) and have other risk factors (i.e. family history, heart disease, high cholesterol levels, etc.) (2).

Self-testing is an important part of controlling diabetes by allowing patients to monitor their blood sugar levels at home and taking the necessary steps to avoid further complications with the condition. Many commercially available glucometers are available to patients to help monitor their sugar levels and determine the appropriate care for the existing condition such as diet, exercise, and how much medications (insulin and glucose-lowering pills) to take (4). Careful monitoring using glucometers ensures that the patients' blood sugar levels remain within the target range and provides important information about trends in blood sugar levels as part of insulin therapy or treatment plan.

Most modern hand-held "finger-stick" glucometers work through enzymatic oxidation of glucose by glucose oxidase. The first generation glucometers contain a photometric sensor to measure the current of the glucose oxidation reaction. D-glucose is oxidized to gluconic acid and hydrogen peroxide via glucose oxidase. The hydrogen peroxide reacts with aminoantipyrine and p-hydroxysulfonate in the presence of peroxidase to form a highly colored quinoneimine product [Figure 1] (5). The meters measure the amount of light reflected or absorbed by the dye-containing surface of the meters to determine the intensity of the color change (photometric measurement.) The intensity of the color change is proportional to the concentration of glucose in the blood sample. The newer generation of glucometers incorporates an electrochemical cell within the device to measure the integrated current produced by the glucose oxidation reaction (electrochemical measurement) (3). D-glucose in the blood sample is oxidized to gluconolactone by the enzyme Glucose Oxidase (GOD) found in the test strip. The reduced enzyme is then re-oxidized by ferrocyanide ion causing a change in the electrical current that indicates the amount of glucose concentration in the sample [Figure 2.] Most glucometers use glucose oxidase as the primary oxidizing enzyme; however, some glucometers use strips with different enzymes such as glucose dehydrogenase that carry out the same principle (3).

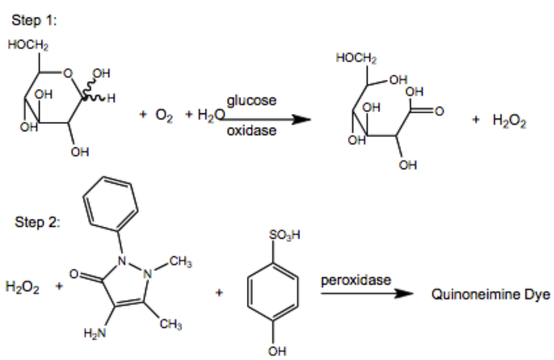

Figure 2: Glucose Oxidation

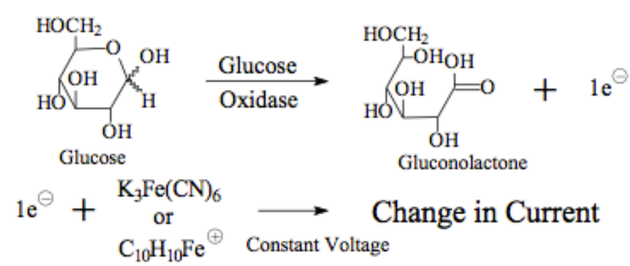

Figure 1: Electrochemical Reaction Within Glucometers

Glucose oxidation reaction is dependent on various variables such as enzyme concentration, glucose concentration, cofactor concentration, time, pH and temperature (5). Temperature and the verification of the calibration of the instrument are the only two variables that most users have control of when using a commercial glucometer. The glucose oxidase lab examines the chemistry behind the use of glucometers specifically at the effects of temperature on the enzymatic reaction of glucose and how a standard curve calibration of varying glucose concentrations can quantitate unknown glucose concentrations in the sample. A second experiment looks at the functioning component of the glucometer including its accuracy and precision, user variation and differences in the design of the glucometers or test strips that may glucose reading (3). It's important to know the chemistry behind how glucometers work in order to determine which ones are appropriate to use and recommend to patients with diabetes for self-monitoring purposes. The labs provide a better understanding and evaluation of glucometers currently available in the market, to become more familiar with the instruments and to know the appropriate similarities and differences between each one.

EXPERIMENTAL

Glucose Oxidase Lab

Reagents –

Glucose (Trinder) reagent consists of 4-Aminoantipyrine (0.5 mmol/L), p-Hydroxybenzene Sulfonate (20 mmol/L), Glucose oxidase from Aspergillus niger (15,000 U/L), Horseradish peroxidase (10,000 U/L), Buffer (pH 7.0 +/- 0.1), Stabilizers and Fillers. The Trinder reagent is a composite of all the components necessary to carry out the enzymatic reactions involved in the assay. 3.00 ml of glucose reagent (Trinder) is added to each of the thirteen test tubes to carry out the reactions. Equal concentrations of the glucose reagent are added to ensure standardization of the glucose samples and enough reagents are used to complete the enzymatic reactions. Glucose oxidase converts glucose into gluconic acid and hydrogen peroxide. The hydrogen peroxide in the presence of 4-Aminoantipyrine and p-Hydorxybenzene Sulfonate will oxidize to form a quinoneimine product via the horseradish peroxidase enzyme. The quinoneimine dye is used to determine the proportionality of the concentration of glucose in the sample. The buffer, stabilizers and fillers are additional components added to the mixture to maintain the appropriate pH to carry out the oxidation reaction and maintain the stability of the enzyme and products formed.

Glucose standards come in six different concentrations: 25, 50, 75, 100, 150, and 200 mg/dL of glucose. Each standard consists of 0.1 M sodium phosphate buffer with pH 7.0. The buffer is used to maintain the pH at appropriate levels to undergo the enzymatic reaction. 40 μL of deionized water (blank), varying standard solutions, or unknown sample are added at one minute intervals to the appropriately labeled test tubes 1 through 9 [Figure 3] [Table 1]. The test tubes are allowed to incubate for ten minutes to allow sufficient time for the reagents to react before the absorbance (A) of each tube is read on the spectrophotometer. Repeat the latter steps for the test tubes 10 through 13 for the ice bath and heated block allowing time for incubation before measuring the absorbance of each.

Deionized water (blank) is used as a filler and control for zero glucose concentration in the sample and is applied to the standard curve.

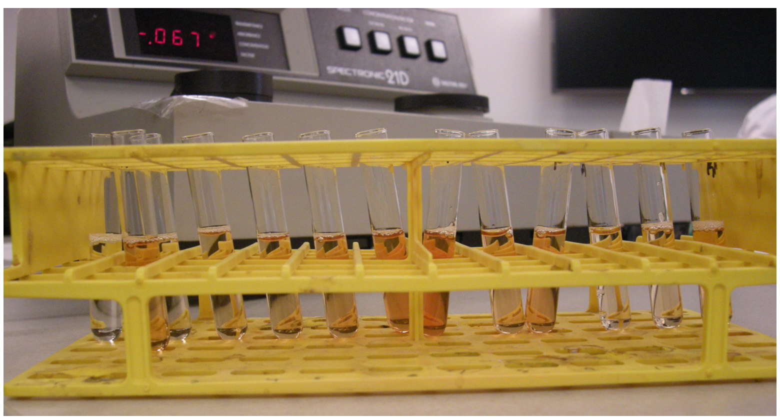

Figure 3: Test Tubes Containing the Reagents and Standard Solutions or Unknowns

Table 1: Standard Solutions of Glucose or Unknown in Appropriate Test Tubes

Tube #	[Glucose], mg/dL	Temperature (Celsius)
1	Blank	20.55
2	25.0	20.55
3	50.0	20.55
4	75.0	20.55
5	100.0	20.55
6	150.0	20.55
7	200.0	20.55
8	Unknown2	20.55
9	Unknown1	20.55
10	Unknown2	0
11	Unknown1	0
12	Unknown2	37.5
13	Unknown1	37.5

Standard curve –

Varying concentrations of glucose are calibrated together to form the standard curve that is then used to determine the concentration of glucose of the unknown sample.

Unknown sample –

The unknown samples contain a specific concentration of glucose that's determined by forming a standard curve and matching the spectrometric output to the curve. Two unknown samples are given in this lab each having its own glucose concentration and are tested in duplicates (have two test tubes to ensure consistency.) The unknown samples are tested in three different temperatures: room temperature (20.55^{o}C), 0^{o}C and 37.5^{o}C each running in duplicates for a total of six samples.

Other instruments –

Glass test tubes, pneumatic pipets are used to transfer the reagents into the proper test tubes, parafilm to mix the reagents prior to measuring with the spectrophotometer (Spectronic 21D) measuring at 505 nm to formulate the standard curve calibration. Additionally, a heated block and ice bath are used to alter the temperatures in which the enzymatic reactions can undergo. One of the variables that users of commercially manufactured glucometers have control over is temperature so it's important to see how different temperatures can affect the overall calibration curve of various unknown concentrations.

Glucometers Lab

Glucometers –

A comparison between two different blood glucose monitoring systems that are commercially available in the market: Accu-Check Advantage (Model No. 8503364095) and One Touch Ultra (Model No. SLDC14DGT.) One Touch Ultra is specific to glucose because it uses glucose-dye-oxidoreductase (GOD) to carry out the oxidation reaction of glucose. Accu-Check Advantage, on the other hand, uses dehydrogenase instead of oxidase to oxidize glucose and is not glucose-specific. Certain glucometers like Accu-Check Advantage cannot distinguish between glucose, maltose or other sugars and are at greater risk of providing false glucose readings. These glucometers usually indicate a higher than normal "glucose" reading because they can't differentiate between glucose and the other sugars in the blood sample. Patients who are receiving EXTRANEAL (icodextrin) peritoneal dialysis solution should not use glucometers that are non-glucose specific because it can lead to falsely elevated glucose readings, inaccurate insulin dosing, and possible health problems such as hypoglycemia (3).

Test Strip –

Each glucometer uses a specific test strip that only works for that particular brand. Accu-Chek Advantage test strips lot number is 548139 and expire 12/31/2005. The description on the container indicates the following: Accu-Chek Comfort Curve for Whole Blood. Used for Accu-Chek Advantage meter and Accu-Chek Complete Meter. The lot number for One Touch Ultra test strips is 3078441 and expires 5/2012 with a control range of 102 – 136 mg/dL and expires 3/2011.

Display and System Check –

Verify that the glucometer is working correctly by making sure the instrument is correctly coded with the appropriate test strip and that the display is working properly. For the Accu-Chek Advantage, a blinking drop of blood on the monitor indicates that the glucometer is ready to use when the appropriate test strip is placed in the slot.

Accuracy and Precision of System –

Determine if the blood glucose reading is within acceptable range as stated on the glucose test strips by using the appropriate glucose standards for each brand of glucometer. The control solution for Accu-Chek Advantage is called Accu-Chek Comfort Curve (lot no. 92380) with Level 2 (HI) control of 4 ml and expires 08/26/2011 with a range of 286 – 388 mg/dL. The One Touch Ultra's control solution (lot no. 9A2A51) has an expiration date of 3/2011. Two drops (~20 μL) of the glucose standard solution is transferred to the finger and then applied to the test strip (siphoning. (LAB) Multiple glucose readings are taken with the test strips being placed right side up and upside down to accurately reflect that patients may not follow the manufacturers' directions exactly. The correct placement of the test strips inside the glucometers ensures accurate glucose readings and to make sure that the results are consistent with patients' base values. Additional readings on additional strips are taken with the same glucose stand solution to determine the precision of the readings and whether they are within acceptable range as indicated on the test strip codes. For one strip, only 5 μL (~1/2 a drop) of glucose test solution is applied to the strip to see if a small volume of "blood" would still reflect a reading or if a minimum volume is needed before an accurate measurement can be taken.

Variables That May Impact Glucose Readings –

Certain variables such as patient negligence and not following the manufacturers' recommendations can lead to inaccurate glucose readings and measurements. Manufacturers recommend that test strips be stored in tight containers at room temperature and away from extreme heat or cold and that glucose control solutions should only be used for specific brands that they are specified for (3). An evaluation of the effect of a strip that's been exposed to air for 24 hours and how using different glucose control solutions on different brands of glucometers can affect glucose measurements and the accuracy of the reading. The One Touch glucose standard solution is used for the Accu-Chek Advantage and the Accu-Chek Advantage is used for the OneTouch Ultra test to determine whether or not glucometers require specific control solutions in order to work.

Glucometer Enzyme Type Affecting Glucose Reading –

Some glucometers are glucose-specific while others are not which can lead to false elevated readings of "glucose." Glucometers that use GOD (glucose oxidase) to carry out the enzymatic reactions are normally specific for glucose while those that use glucose dehydrogenase are non-specific for glucose and cannot differentiate between glucose and other sugars in the sample leading to possible false readings. Two drops (~20 μ) of the spiked-maltose control solution instead of the normal glucose solution is used to determine whether or not the glucometers would give an elevated or normal glucose reading. An elevated reading above the acceptable range would indicate that the glucometer cannot differentiate between the various sugars in the sample and would not be appropriate to use for patients under dialysis.

RESULTS

Glucose Oxidase Lab

Determine if temperature differences affect enzymatic reactions of glucose (glucose oxidation)

Varying concentrations of glucose are used to calibrate the standard curve in order to determine the concentrations of the two unknowns given (Unknown 1 and Unknown 2.) Equal volumes (0.04 ml) of each of the six standard glucose concentrations and appropriate unknown solutions (Unknown 1 and Unknown 2) are added to the designated test tubes at 20.55°C (room temperature) and allowed to react with the other reagents for 10 minutes. After the incubation time, the absorbance of all the test tubes are measured by the spectrophotometer and the results are given in Table 2.

Table 2: Absorbance Results

Tube #	[Glucose], mg/dL	Temperature (Celsius)	Absorbance
1	Blank	20.55	0
2	25.0	20.55	0.089
3	50.0	20.55	0.17
4	75.0	20.55	0.252
5	100.0	20.55	0.401
6	150.0	20.55	0.698
7	200.0	20.55	0.938
8	Unknown2	20.55	0.332
9	Unknown1	20.55	0.507
10	Unknown2	0	0.119
11	Unknown1	0	0.162
12	Unknown2	37.5	0.367
13	Unknown1	37.5	0.522

0.04 ml of the each of the unknowns are added to the corresponding test tubes at appropriate temperatures (0°C and 37°C) to determine the effects of temperature on the oxidation of glucose. Again, the reagents are allowed to incubate for 10 minutes before the absorbance is measured (Table 2.)

Standardization of the glucose curve to estimate the concentration of the unknown (Method 1)

The standard concentrations of glucose can be calibrated appropriately according to their absorbance results to form a calibration curve, which can then be used to figure out the concentrations of the unknown samples. Each standard value can be used as a proportionality constant to estimate the unknown, but these values need to be corrected for possible error in the evaluation of the unknown samples.

Glucose Concentration (mg/dL) of Sample =

$$(A_{sample} - A_{blank})/(A_{standard} - A_{blank}) \text{ x Concentration of Standard}$$

The latter equation involves the use of standard values for both the concentrations and absorbance of the standard solutions to figure out the concentrations of the unknowns.

A_{sample}: Absorbance value of the unknown given

A_{blank} = 0 (if the spectrophotometer is zeroed with deionized water and reagent)

$A_{standard}$: Absorbance value of the standard solution

Each test tube (8-12) containing the unknowns has to be calculated with each of the six standard glucose solutions from the above equation and then averaged out. It’s important to note that since each standard concentration contains a small margin of error, the value for the unknown concentration is different for each. The average is taken to provide a standardization of the all the values received for that particular unknown in reference to all the standards used in the calculations [Table 3.] Standard deviation calculates the spread or dispersion of the overall data specifically pertaining to the mean or average value. A low standard deviation indicates that the data given is concentrated around the mean while a high SD implies greater spread of data. Hence, a low SD value would give a better picture of the data and its distribution in terms of the average that's calculated for all the standards used in the calculation.

Table 3: Calibrated curve values and concentrations of unknown samples

	Method 1	Glucose concentration of sample (mg/dL)							
Standard Concentrations		**25 mg/dL**	**50 mg/dL**	**75 mg/dL**	**100 mg/dL**	**150 mg/dL**	**200 mg/dL**	**Average**	**Standard Deviation**
	Tube 8 (Unknown 2)	93.258	97.647	98.81	82.793	71.347	70.789	85.774	12.71605753
	Tube 9 (Unknown 1)	142.416	149.118	150.893	126.434	108.954	108.102	130.9862	19.4191151
	Tube 10 (Unknown 2)	33.427	35	35.417	29.676	25.573	25.373	30.74433	4.557992087
	Tube 11 (Unknown 1)	45.506	47.647	48.214	40.399	34.814	34.542	41.85367	6.204667942
	Tube 12 (Unknown 2)	103.09	107.941	109.226	91.521	78.868	78.252	94.81633	14.05656164
	Tube 13 (Unknown 1)	146.629	153.529	155.357	130.175	112.178	111.3	134.8613	19.99331823

The averages of the two unknowns (Unknown 1 and Unknown 2) are not exact and fall within a wide range according to the calculations. This is the due to the margin of error of the standards that can yield some discrepancy with the concentrations as well as the standard deviation value of each in relations to the average values. According to the results, the average concentration values of Unknown 1 are consistently greater than the values of Unknown 2 at all three temperatures. Table 4 provides a better picture showing the relationship between temperatures and the calculated values of the two Unknowns in question as well as the absorbance values for each.

Table 4: Glucose Concentrations of Unknowns Using Method 1

Tube #	[Glucose], mg/dL	Temperature (Celsius)	Absorbance	
1	Blank	20.55	0	
2	25.0	20.55	0.089	
3	50.0	20.55	0.17	
4	75.0	20.55	0.252	
5	100.0	20.55	0.401	
6	150.0	20.55	0.698	
7	200.0	20.55	0.938	**Method 1**
8	Unknown2	20.55	0.332	85.77
9	Unknown1	20.55	0.507	130.99
10	Unknown2	0	0.119	30.74
11	Unknown1	0	0.162	41.85
12	Unknown2	37.5	0.367	94.82
13	Unknown1	37.5	0.522	134.86

The use of a linear regression to determine the concentrations of the unknowns (Method 2)

A graphical method is another useful way to determine the concentrations of the unknowns by providing a linear regression line of the data to show the relationship between glucose concentration and absorbance. The x-axis shows the glucose concentrations (mg/dL) of the six standards of measurement with varying concentrations of glucose (Test tube 1-7) at room temperature. The y-axis indicates the absorbance values of the standards that are measured with the spectrophotometer in the experiment. The values of the standard glucose concentrations and their corresponding absorbance values are graphed accordingly to form a linear regression [Graph 1.] From the linear regression, an equation of the line is given as well as the R^2 value that accounts for the discrepancy or variability of the data along the line.

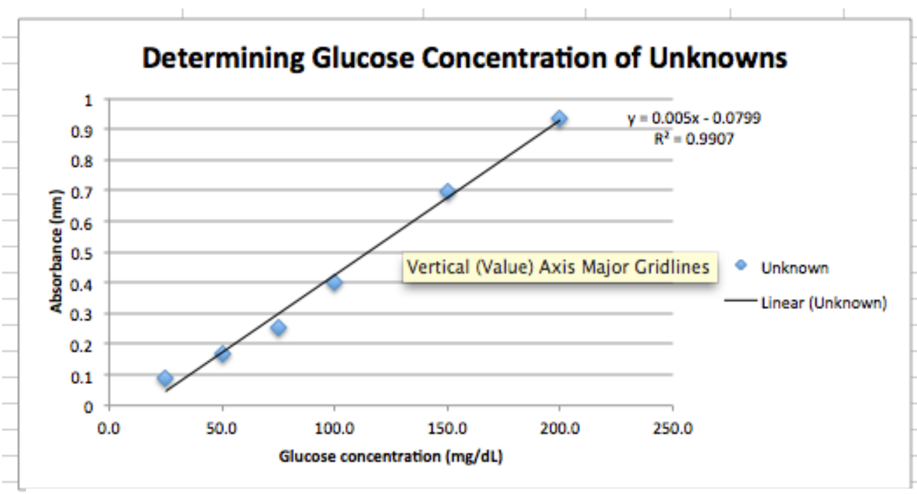

Figure 4: Linear Regression Line of Standard Solutions and Absorbance Values

The given equation y = 0.005x – 0.0799 with "y" being the absorbance value and "x" being the glucose concentration can be used to calculate the glucose concentrations of the two unknown samples according to their absorbance values (Table 5) that are given from the spectrophotometer readings. Similar to the results from Method 1, the glucose concentrations for Unknown 1 and 2 are not consistent with each other which may be due to the differences in temperatures. However, it can be inferred from the given data that Unknown 2 has a lower glucose concentration than Unknown 1 which is consistent across all three temperatures.

Table 5: Glucose Concentrations of Unknowns Using Linear Regression

	Method 2		
y= 0.005x - 0.0799		**Absorbance**	**Glucose concentration (mg/dL)**
y= absorbance	Tube 8 (Unknown 2)	0.332	82.38
x= glucose concentration	Tube 9 (Unknown 1)	0.507	117.38
m=slope = 0.005	Tube 10 (Unknown 2)	0.119	39.78
	Tube 11 (Unknown 1)	0.162	48.38
	Tube 12 (Unknown 2)	0.367	89.38
	Tube 13 (Unknown 1)	0.522	120.38
			slope (m): 0.005
			y-intercept (b): -0.0799

Table 6 shows the values of the glucose concentrations of the two unknown samples derived from both Method 1 and 2. Although the values are not exact when compared, they do fall within a relative range of each other with a slight margin of error.

Table 6: Glucose Concentrations of Unknowns From Method 1 and Method 2

Tube #	[Glucose], mg/dL	Temperature (Celsius)	Absorbance		
1	Blank	20.55	0		
2	25.0	20.55	0.089		
3	50.0	20.55	0.17		
4	75.0	20.55	0.252		
5	100.0	20.55	0.401		
6	150.0	20.55	0.698		
7	200.0	20.55	0.938	**Method 1**	**Method 2**
8	Unknown2	20.55	0.332	85.77	82.38
9	Unknown1	20.55	0.507	130.99	117.38
10	Unknown2	0	0.119	30.74	39.78
11	Unknown1	0	0.162	41.85	48.38
12	Unknown2	37.5	0.367	94.82	89.38
13	Unknown1	37.5	0.522	134.86	120.38

Glucometer Lab

There are numerous commercially available glucometers available in today's market that may carry out similar functions, but they also have variation in testing and the specific components used to measure the glucose readings. The glucometer lab looks at these differences and determines whether certain glucometers are better than others and examines the overall design and biochemistry behind them. Tests results are given in Table 7 and Table 8.

Verify that the instruments are working correctly and correctly coded with the appropriate test strips before testing can be done

The Accu-Chek Advantage glucometer use a specific test strip that only works for that particular brand. The time and date of testing are input into the monitor before coding can be done with the test strip by turning on the power button, and getting to the display board containing the time/date and other information pertaining to the glucometer. A "display check" to see if the meter is ready to be tested is performed by coding it with the test strip. The test strip contains a code (Code 139) that matches the code to be used on that particular glucometer model ensuring that further testing can take place. A test strip is inserted in the glucometer according to the manufacturer's direction. A blinking drop of blood appears on the display monitor indicating that the instrument is ready to be used and that the test strip is compatible with that particular glucometer.

The OneTouch Ultra glucometer is coded by matching the code on the meter display with the code on the testing strip vial code associated with it to ensure compatibility. In order to perform this test, the meter needs to be turned off. A OneTouch Ultra test strip is inserted into the appropriate slot to turn on the meter and various segments of the display are shown. A code number appears on the display for three seconds and this code is matched with the code on the strip vial. Once the system check is done, the meter is ready to use. Also, the display monitor should also have an image of a bolded hypen (**----**) and mg/dL indicating that the instrument is ready.

Perform the appropriate glucose reading using the glucose standards (control solution) provided by the manufacturer to determine the accuracy and precision of the system

Prior to measuring the glucose mixture using the glucometer (both the Accu-Chek and One Touch Ultra glucometers), a display check is performed to ensure that proper controls are taken. A test strip is inserted right side up into the glucometer and upside down and results recorded (Test #0). Since no glucose has been added to the strip, the display does not indicate a numerical value, but shows the blinking drop of blood (need to add to the control solution.) The blinking drop of blood does not appear when the strip is inserted upside down because it's not properly inserted for the system to work.

Two drops (~20 μL) of the glucose standard solution are placed on the finger and transferred to the test strip that has been properly inserted into the glucometer (Test #1). A glucose reading is obtained with the given amount of control solution on the glucometer as 271 mg/dL (15.04 mmol/L) for the Accu-Check monitor and 138 mg/dL (7.66 mmol/L) for the One Touch Ultra. Once the results of the readings have been recorded, each of the test strip is removed from the glucometer and reinserted after one minute (60 seconds) and results recorded. This test determines whether patients can read an old test strip once it has been removed from the glucometer or that a fresh strip is required for a new reading. In both cases, the monitor indicates an ERROR message for both of the glucometers meaning that the strips cannot be used once they have been removed from the insert.

Two additional tests similar to Test #1 using ~20 μL of the glucose standard solution are done (Test #2 and Test #3) to determine if the results are consistent with each other (precision test) and if the results fall within the acceptable range established by the manufacturers for that particular control solution. The acceptable range for the Accu-Chek control solution is 286-388 mg/dL and 20-600 mg/dL for the One Touch Ultra control solution. The glucose readings for the Accu-Check using 20 μL of the control solution are below the acceptable range (has a more narrow range.) The readings are for the One Touch Ultra are within the appropriate range (has a much wider range.)

Certain glucometers require a minimum volume of blood in order to work and produce a reading. Sometimes patient do not use enough blood which can interfere with the readings or give inaccurate results that are not reflective of the actual glucose levels in the body. Thus, it's important to use enough blood in order to ensure a proper reading on the glucometers. Test #5 uses only 5μL (~ ½ a drop) of the glucose test solution instead of the normal ~20 μL.(a 75% decrease in the volume of blood.) The glucose readings for both the Accu-Check and One Touch Ultra monitors are not exactly the same as the other six readings that use the full drop, but are still very close (only a slight difference.) This indicates that both of the glucometers are able to detect the small amount of the samples and give an appropriate reading of each. Having a larger volume of "blood" or control solution ~20 μL (2 drops) ensures that a reading can be obtained and produce consistent results, but these glucometers are manufactured in such a way that even a small volume of blood or solution can still be detected and produce similar results.

User variation may affect the proper use of the glucometers as well as the accuracy of the results.

Manufacturers recommend that test strips be stored in tightly capped containers away from extreme temperatures and only use glucose control solutions that are specific for that particular brand of glucometer. Thus, exposing the strips to air could decrease their effectiveness and cause inaccurate glucose measurements.

Test strips that have been left exposed for 24 hours are tested with the Accu-Chek and OneTouch Ultra glucose standard solutions (~20 μL) and results recorded (Test #5.) The glucose readings increase significantly for both of the glucometers putting the Accu-Chek glucose reading into the control range set up by the manufacturer (286-388 mg/dL.) The glucose reading for the One-Touch Ultra, however, is slightly above the range (139 mg/dL) so it's considered invalid.

Using an inappropriate standard glucose solution that's not specifically for a certain brand of glucometer (not recommended by the manufacturer) can also produce inaccurate results. A fresh test strip (not exposed for 24 hours) is tested using a different brand of control solution for each of the glucometers and the results recorded (Test #6.) The One Touch glucose standard solution is used for the Accu-Chek Advantage monitor while the Accu-Check stand solution is used for the One Touch glucometers. When a different control solution is used to test the glucometers, the results increase drastically (295 mg/dL for the

Accu-Chek and 347 mg/dL for the One Touch Ultra.) The use of a different control solution puts the glucose reading within the normal control range for the Accu-Chek monitor, but causes the One Touch instrument to be way above the normal range.

Some glucometers require a specific enzyme type in order to produce accurate results.

Some glucometers that use glucose oxidase (enzyme in the test strip) are highly sensitive and glucose-specific. Other glucometers that use glucose dehydrogenase are less sensitive because they cannot distinguish between glucose, maltose and other sugars in the body which can provide falsely elevated “glucose” readings. Thus, all the sugars are assumed to be glucose by the glucometer that can lead to a higher reading of glucose and inappropriate dosing by the patient. For test #7, a spiked-maltose control solution is used instead of the manufacturers’ control glucose solution to see if the glucometer is able to distinguish between the glucose and other sugars. Accu-Chek Advantage uses GDH-PQQ as its main enzyme so it’s not glucose-specific while One Touch Ultra uses GOD (glucose oxidase) and is more specific for glucose. This is reflected in the glucose readings for the two instruments. The Accu-Chek Advantage has a much lower reading (249 mg/dL) for the glucose (below the range) than expected using the maltose-spiked solution while the reading with the One Touch Ultra glucometer yields a more appropriate result (139 mg/dL) that’s consistent with the other previous readings. Test #8, #9, and #10 compares the results with other groups working with the same meter type to determine the consistency and precision of the data.

Table 7: Results of the Accu-Chek Advantage Tests

Glucometer Model:	Accu-Chek Advantage		Glucometer No.	8503364095	
Control Solution No.	92380		Test Strip Lot No.	548139	
	Accu-Chek Comfort Curve Level 2 (HI) Control 4 ml			Expires: 12/31/05	
	Expires: 08/26/11			Accu-Chek Comfort Curve for Whole Blood. Used for Accu-Chek Advantage meter and Accu Chek Complete Meter. 50 pack	
Strip Test	**Glucose Sample**	**Special Conditions**	**[glucose] mg/dL**	**[glucose] mmol/L**	**Other Observations**
#0	0	Strip rightside up	n/a	n/a	Code 139 (match). Get a strip symbol with blinking drop of blood
#0	0	Strip upside down	n/a	n/a	Asks for strip. No blinking drop of blood.
#1	20 µL	none	271	15.04252873	
#1	20 µL	reread after 60 s	Error	n/a	
#2	20 µL	none	240	13.32179666	
#3	20 µL	none	252	13.98788649	
#4	5 µL	none	270	14.98702124	
#5	20 µL	exposed 24 h	328	18.20645543	
#6	20 µL	different control	295	16.37470839	
#7	20 µL	maltose-spiked	249	13.82136403	
#8	20 µL	from other group	340	18.87254526	
#9	20 µL	from other group	343	19.03906772	
#10	20 µL	from other group	315	17.48485811	

Table 8: Results of the One Touch Ultra Tests

Glucometer Model:	One Touch Ultra by Lifespan		Glucometer No.	SLDC14DGT
Control Solution No.	9A2A51		Test Strip Lot No.	3078441
				Expires: 5/12
	Expires: 08/26/11			Control Range: 102-136 mg/dL
Strip Test	**Glucose Sample**	**Special Conditions**	**[glucose] mg/dL**	**[glucose] mmol/L**
#0	0	Strip rightside up	n/a	n/a
#0	0	Strip upside down	n/a	n/a
#1	20 µL	none	138	7.660033078
#1	20 µL	reread after 60 s	Error	n/a
#2	20 µL	none	138	7.660033078
#3	20 µL	none	134	7.438003134
#4	5 µL	none	137	7.604525592
#5	20 µL	exposed 24 h	139	7.715540564
#6	20 µL	different control	347	19.26109767
#7	20 µL	maltose-spiked	139	7.715540564
#8	20 µL	from other group	124	6.882928273
#9	20 µL	from other group	118	6.549883357
#10	20 µL	from other group	126	6.993943245

Table 9: Results of the Readings Using the Manufacturer's Directions and Comparison With Other Groups (Accu-Chek Advantage)

		[Glucose] mg/dL	[Glucose]mmol/L
Test Strips #1, #2, #3 (n=3)	**Average:**	254.3333333	14.11740396
	Std Dev:	15.63116545	0.867646699
	%CV:	6.145937	6.145937
		[Glucose] mg/dL	[Glucose]mmol/L
Test Strips #1, #2, #3, #8, #9, #10 (n=6)	**Average:**	293.5	16.29144716
	Std Dev:	45.08991018	2.502827561
	%CV:	15.36283	15.36283

Table 10: Results of the Readings Using the Manufacturers' Directons and Comparison With Other Groups (One Touch Ultra)

		[Glucose] mg/dL	[Glucose]mmol/L
Test Strips #1, #2, #3 (n=3)	**Average:**	136.6666667	7.586023097
	Std Dev:	2.309401077	0.128189048
	%CV:	1.689805666	1.689805666
		[Glucose] mg/dL	[Glucose]mmol/L
Test Strips #1, #2, #3, #8, #9, #10 (n=6)	**Average:**	129.6666667	7.197470694
	Std Dev:	2.309401077	0.457277485
	%CV:	1.781029108	6.353308044

DISCUSSION

The proper measurement of glucose in biological fluids is an important component in diagnosing diabetes because it examines the abnormal carbohydrate metabolism in the body. Diabetes is characterized by abnormally high concentrations of glucose in physiological fluids due to the body's inability to break down the glucose to useable forms of energy (5). Many commercial glucometers are currently available in the market to measure blood glucose levels, but there are significant variations in each that need to be taken into account. Glucometers work by way of enzymatic oxidation of glucose through a variety of enzyme types such as glucose oxidase or glucose dehydrogenase. Different enzymes process sugars in the body differently, some are more glucose-specific than others, hence appropriate glucometers are needed depending on the user's medical condition. The labs examine the enzymatic variables and factors that may affect enzymatic oxidation of glucose and the variations in the different glucometer types.

Most enzymatic reactions such as the oxidation of glucose are dependent on numerous enzymatic variables such as enzyme concentration, glucose concentration, cofactor concentration, time, pH and temperature (5). When using the commercial glucometers, the only variables that the users have much control over are the temperature and verification of the calibration of the instrument. The glucose oxidase lab looks at how these variables specifically the temperature and the standard curve calibration can be used to determine the glucose concentrations of a sample and the degree of the concentrations (high or low.)

Two different methods are used in the experiment to determine the glucose concentrations of the two unknown samples given. The first method uses a standard curve calibration while the second method uses a linear regression to determine the appropriate concentrations. The calibration of the standard curve uses a specific equation, varying concentrations of glucose standards, and absorbance values to estimate the concentrations of the unknowns. It's also important to take into account the effect of temperature on the final results and the discrepancy between the values. At room temperature, Unknown 1 is calculated to be 130.99 mg/dL and 85.77 mg/dL for Unknown 2. At 0°C, the concentrations decrease significantly with Unknown 1 being 41.85 mg/dL and 30.74 mg/dL for Unknown 2. At 37.5°C, the value for Unknown 1 is 134.86 mg/dL and 94.82 mg/dL for Unknown 2. The glucose concentrations are not consistent due to the varying of temperatures in which the reactions take place. At low temperatures, the calculated glucose concentrations of the unknown samples are much lower than at normal temperatures due to the fact that the glucose oxidation reaction might be slower and take longer for the enzyme to fully react with the other reagents. At low temperatures, the enzymes may not react as fast to break down the glucose and form the highly colored quinoneimine product so the solution is more lightly colored. The intensity of the colored product formed is directly proportional to the concentration of the glucose in the sample. At high temperatures, however, the glucose concentrations of the unknowns are much higher because more glucose is reacted with the glucose oxidase (enzyme speeding up) so more of the quinoeimine product is produced (darker color.) However, if the temperature gets too high, it can denature the enzyme and prevent it from breaking down the glucose in the reaction so less product would be produced as a result. Temperature is an important element that needs to be taken into account when using a glucometer to prevent getting any inaccurate results or readings. Test strips should not be placed in extreme temperatures because the enzymes can become denatured or ineffective when measuring the blood glucose. Thus, all test strips should be placed in a tightly-sealed container at room temperature to maintain their effectiveness.

The second method uses a linear regression line to figure out the glucose concentration of the unknown samples. The linear regression line shows the relationship between glucose concentration using the standard solutions and absorbance (measured by the spectrophotometer.) The equation of the linear regression line can be used to determine how much glucose is in the unknown samples by their absorbance values. Although this is an appropriate method to use to figure out the glucose concentrations, it's also important to consider the variability of the data along the line and account for any discrepancy that might occur. The results for this particular experiment have very consistent numbers so the variability is not as great with a R^2 value of 0.9907 (close to 1.) Hence, there should be reduced discrepancy of the final glucose concentrations of the two unknown samples. Like the first method, the values of the Unknown 1 and Unknown 2 are not consistent with each other due to the differences in temperatures. However, when the values of the concentrations are compared across Method 1 and 2, the values differ slightly but still within approximate range. Although the exact concentrations cannot be known for the samples (only the averages can be measured), it can be concluded based on the results that Unknown 1 has a higher glucose concentration than Unknown 2 (consistent across all three temperatures.) Again, this second method emphasizes the importance of temperature and their effects on glucose readings that should be taken into account when exposing the test strips to high or low temperatures.

Test strips are highly sensitive to temperature and other factors that may reduce their effectiveness and ability to measure blood glucose in the body. The accuracy of the glucose measurements is highly dependent on the proper functioning of the test strips and the glucometer that's associated with them. Any variation in the glucometer or test strip such as using a different control solution, exposing the strips to air for more than 24 hours or simply not using enough sample for testing can compromise the glucose results/readings. The glucometer lab looks at the various factors that may affect the sensitivity of the test strips and user variations that may impact the use of the glucometers as well as the measurements. Prior exposure of the test strips to air and light destroy the enzymes on the test strips so they become ineffective and produce inaccurate results and bad data. Surprisingly, the glucose readings are still within the acceptable range of the control solution (within 2 SD.) However, this can be attributed to the expired products providing inconsistent and inaccurate results or that the enzymes on the strips haven't been fully denatured yet. Additionally, when only 5 μL of the volume of solution is used, the results are still consistent and within 2 SD o the first reading and within the acceptable range some (if error is taken into account.) However, when a different control is used, the glucose reading increase significantly to go outside the acceptable range for the One Touch Ultra but still within range for the Accu-Chek system.

The lab also examines the effect of the control solutions recommended for use by the manufacturers to determine the precision and accuracy of the glucometer readings (whether they are within the acceptable range set up by the manufacturers.) The acceptable range for each control solution is found in the bottle lab indicating whether the testing is more high (HI) or low (LO) levels of glucose. The results for the Accu-Chek Advantage glucometer tend to be below the acceptable range (286-388 mg/dL) for that particular HI (high) control solution. This is probably due to the fact that both the control solutions and test strips are expired which can yield inaccurate results. The test strips have been expired since 12/31/05 and the control solution expired last year (08/26/11.) However, the manufacturers recommend that all control solutions and test strips that are expired or opened more than 3 months ago need to be discarded because they can compromise the glucose measurements and put them out of range. Groups that have their control solutions and test strips not yet expired have more consistent results that are within the acceptable range (Test #8, #9, #10.)

The One Touch Ultra glucometer also has expired control solutions (3/11), but their test strips are valid until 5/12 of this year. However, the values of the glucose readings are still above the normal range. Thus, it's important to use control solutions and test strips that haven't yet expired to do the measurements to ensure accuracy and precision. For the Accu-Chek glucometer, the mean value of the expected range for the glucose control solution is 337 mg/dL and 119 mg/dL for the One Touch Ultra solution. The American Diabetes Association allows the glucose measurements using the control solution to be within 10% of the range (257.4 – 426.8 mg/dL for Accu-Chek and 91.8 – 149.6 mg/dL for the One-Touch Ultra) and within 15% of the reference value. Based on the glucose readings and the 10% error acceptance, both the readings for the Accu-Chek and One Touch Ultra instruments would be within the appropriate range and would be consistent with the American Diabetes Association consensus statement.

Both of the glucometers are designed in such a way that they are easy to use and handled by the users. When users first use these instruments they need to set up the date and time and make sure that the strip codes match the glucometers' codes. A code check ensures that the test strip is compatible with a particular brand of glucometer and produce accurate readings. The system does a pretty good job in letting the patient know if the strip and the monitor aren't compatible by showing the incomplete symbols on the monitor screen. However, if the patients doesn't read the manual beforehand, they would probably have a difficult time figuring out what the symbols mean and what steps are necessary to perform the system and code checks. Therefore, it's important to read the user manual first before doing the initial testing to figure out the meaning of the symbols and how to calibrate the glucometer efficiently. Both the Accu-Chek and One Touch require an initial setup such as the time/date and display check to make sure that all the necessary components are ready to go before further testing can take place. The One Touch Ultra glucometer requires a system check in which users have to put in the code of the test strip prior to doing the testing to ensure compatibility between the strip and the monitor itself. The standard curve for the glucose oxidase assay is entered into the glucose monitoring device by way of using the control solution to determine if the glucose readings and measurements will fall within an acceptable range set up by the manufacturers them. The control solutions test for high or low levels of "glucose" as form of control and standardization.

A comparison of the standard deviations and percent coefficient of variation (%CV) of both of the glucometers indicates the variability of the data obtained from the experiment. For the Accu-Chek system, the average glucose reading using one glucometer is 254.33 mg/dL which is way below the acceptable range given by the manufacturer. However, the SD value is pretty high for this particular value (15.63) so there's a lot of variability in the data obtained. However, when using the data from other groups with the same glucose control solution, the glucose average is much higher (293.5 mg/dL) and within the acceptable range, however, the SD has increased significant (45.09) so there's an increase in the variability of the data obtained. However, the standard deviation and %CV for the One Touch are comparable when comparing the two instruments from the other groups. The SD value is roughly around 2.31, a low value compared to the Accu-Chek system meaning there's less variability in this system and the data is more acceptable in this case.

Different glucometers have their advantages and disadvantages. For the Accu-Chek system, it is easy to use, simple clean-up procedure, long warranty if registered easy-to-navigate website. If I made a call to the 800 number to ask why I received two different readings in a row and wanted to know which was right. I was told that the glucometer might be having some glitches and precision issues. I was told to get rid of that glucometer and get a new one to fix the problem. I didn't think that was an appropriate answer because I thought they were trying to scam me to buy a product. The operator didn't ask for more information and tried to help me figure out the problem instead tried to sell me a new product instead. The disadvantage of this system is not it's restricted to the finger only and not other sites and the difficult to recall prior reading (difficult to navigate the system and know which buttons to push) as well as their poor support system. However, this system would be appropriate for the elderly who have poor vision and severe arthritis, but not for dementia because of the poor memory system. The monitor itself provides a good grip and the numbers are big and easy to read. The One Touch Ultra glucometer is a slimmer model than the Accu-Chek Advantage so would be appropriate for a young student to have on hand (takes up less storage space.) The One Touch glucometer is easy to use and clean up, sample can be taken from other sites and not just the finger, easy to manage website that addresses FAQ's that patients have, and good memory system. The cons for this particular glucometer are that it's difficult to handle since it's so small so an older person would have trouble handling it and that a coding system (putting in the actual code) is required before to testing can begin to ensure compatibility. It's also important to take to consideration that various factors could affect the accuracy of the glucometer such as environmental temperature and humidity, which can degrade the enzymes and make them ineffective in the measurement of glucose.

Certain medical conditions can interfere with the glucose readings such as dialysis. A patient receiving EXTRANEAL (icodextrin) peritoneal dialysis solution causes the metabolism of icodextrin to maltose in the body (in vivo.) Thus, their maltose levels can increase significantly in the body and cause elevated glucose readings if the proper glucometer is not used. Patients who are receiving this treatment should use the One Touch Ultra glucometer because the instrument uses an enzyme that's glucose-specific so the maltose will not interfere with the readings and produce elevated glucose results. Accu-Chek glucometers are non-specific so they will consider the maltose as glucose in the body and provide inaccurate glucose readings. Today's market is filled with hundreds of glucometer types that all do pretty much the same thing, measure a person's blood glucose level oftentimes as part of their diabetes regimen. However, there are slight variations between them that need to be taken into considerations as well as the many residing factors that may influence the final results such as patient health status and environmental conditions.

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