BIOM9420 Clinical Trial Group Report - Group Work Contribution

Team work is one of the graduate attributes that will be developed during this course as it is an important part of the activities of your future career. Working in groups for assessment tasks can be challenging and can potentially lead to unequal contributions to the overall project provided by the individual team members. How this is managed is up to the team members to organise but it is important that the workload is shared appropriately. For this reason your relative individual contribution to the group project will be reviewed and used to moderate individual marks if deemed necessary.

Please complete the following contribution declaration and submit with the assessment.

Abstract		**Introduction**		**Materials and Methods**		**Results**		**Discussion**		**Conclusions**	
Person(s) Responsible	% contribution	Person(s) Responsible	% contribution	Person(s) Responsible	% contribution	Person(s) Responsible	% contribution	Person(s) Responsible	% contribution	Person(s) Responsible	% contribution
Dan	33	Angus	80	Dan	5	Angus	75	Brodie	35	Dan	33
Gilbert	33	Gilbert	20	Gilbert	45	Dan	25	Marco	35	Marco	33
Angus	33			Angus	50			Dan	25	Brodie	33
								Gilbert	5		

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Submission Date	*Course Coordinator/Lecturer*

Clinical Trial
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BIOM9420
Clinical Laboratory Science

Clinical Trial Report

Term 2 2019

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Abstract

The prevalence of diabetes has become a challenge for modern society. Sensi-GO™ technology represents a new generation of glucose urinalysis that is designed to be both quick and analytically inexpensive. This report examines the clinical trial experiment for Sensi-GO™ where urine samples containing glucose were qualitatively identified. However, Sensi-GO™ was unable to conclude quantitatively the glucose concentration of the samples due to invalid results from a potentially flawed procedure. Sensi-GO™ results are discussed in detail concerning its discrepancies, experimental design, comparisons to potential competitors, and advised improvements for any further research.

Table of Contents

1. Introduction

Diabetes is a prevalent metabolic disorder characterised by the body's failure to maintain healthy blood glucose levels. It can cause a range of health complications if left untreated, including death. All types of diabetes are caused by the body's failure to either produce or respond to the hormone insulin, which facilitates the movement of blood glucose into the body's cells. In every case, diagnosis and treatment relies on the ability to measure the level of glucose in the bloodstream. In Australia it is estimated that over 1.2 million adults are living with diabetes [1], meaning there exists a large market for glucose biosensors. Sensi-GO™ is a new biosensing technology based on a novel formulation of glucose oxidase. The two-part reaction produces a dark green coloured compound called ABTSG in the presence of glucose substrate. The current market leader in urinalysis is Multistix®, which can only conduct qualitative tests for glucose concentration. The main advantage of Sensi-GO™ is that the intensity of green is linearly proportional to the concentration of glucose in a measured sample, therefore, the test is quantitative and increases the efficiency of glucose urinalysis. Sensi-GO™ is poised to fill the market space as a more efficient glucose monitor. The Sensi-GO™ test strip is made of absorbent paper on which the patient samples are applied. Next, the Sensi-GO™ compounds are dropped onto the patient sample, which has since spread through the paper. The colour can be measured with a separate piece of equipment such as a mobile phone to obtain the concentration of glucose in the initial sample.

2. Clinical Trial Experiment

This clinical trial experiment aims to measure the effectiveness of Sensi-GO™ reactions in both detecting the presence of glucose in a given patient sample and determining its concentration. The sucrose solution acts as the controlled variable with the expectation of little variation of data. It is expected that the intensity of the green colour is correlated to the concentration of glucose in the sample.

2.1 Materials

The materials required for this experiment are:

- ➢ 10 clean pipettes
- ➢ At least 35 filter paper test strips (50 x 5mm)
- ➢ 10, 15, 20, 25 mM glucose solution
- ➢ 0, 10, 15 mM sucrose solution
- ➢ Part A & B of Sensi-GO™ solution
- ➢ Patient samples
- ➢ Colour Grab application

2.2 Method

Paper strips were laid down in three major groups: the sucrose control group, which had three known concentrations and laid in ascending order. The glucose control had four known concentrations laid in ascending order. The patient group had a column representing each patient. Two identical rows were added below for every group for repeat measurements. The various patient and control samples were prepared in clearly marked containers. The Color Grab application was also loaded.

The control groups were tested first with sucrose to confirm Sensi-GO™ was not sensitive to sucrose. A single drop of the 0 mM solution was placed onto its prepared strip and corresponding repeat strips below, followed by the 10 and 20 mM solutions. The dropping procedure was repeated with Sensi-GO™ part A then by part B. After each application of part B, the reaction was allowed to settle and the colour measurement was taken. This entire section was repeated for the glucose group.

The final group was made from patient samples and its purpose was to test Sensi-GO™'s ability to determine glucose concentration from a series of unknown samples. The same procedure as above was employed.

3. Results

3.1 Sensi-GO™ Results

The RGB values of the glucose solution, sucrose solution and patient samples after reacting to Sensi-GO™ are respectively recorded in *tables 1, 2, and 3* in Appendix F.

Figure 1 and *figure 2* (below) presents the recorded colour of the glucose controls and tested patient samples respectively in terms of the relative values of the RGB values. The equivalent chart for the sucrose control is given in Appendix G.

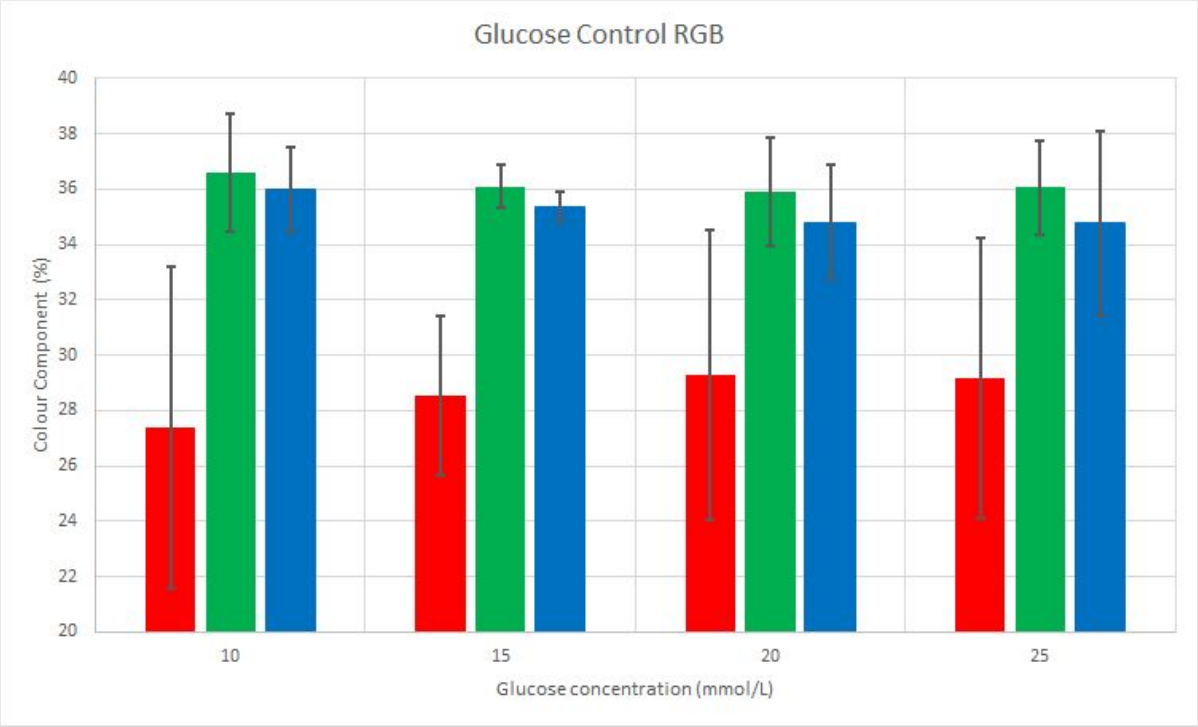


Figure 1 - RGB Values of Glucose Solutions

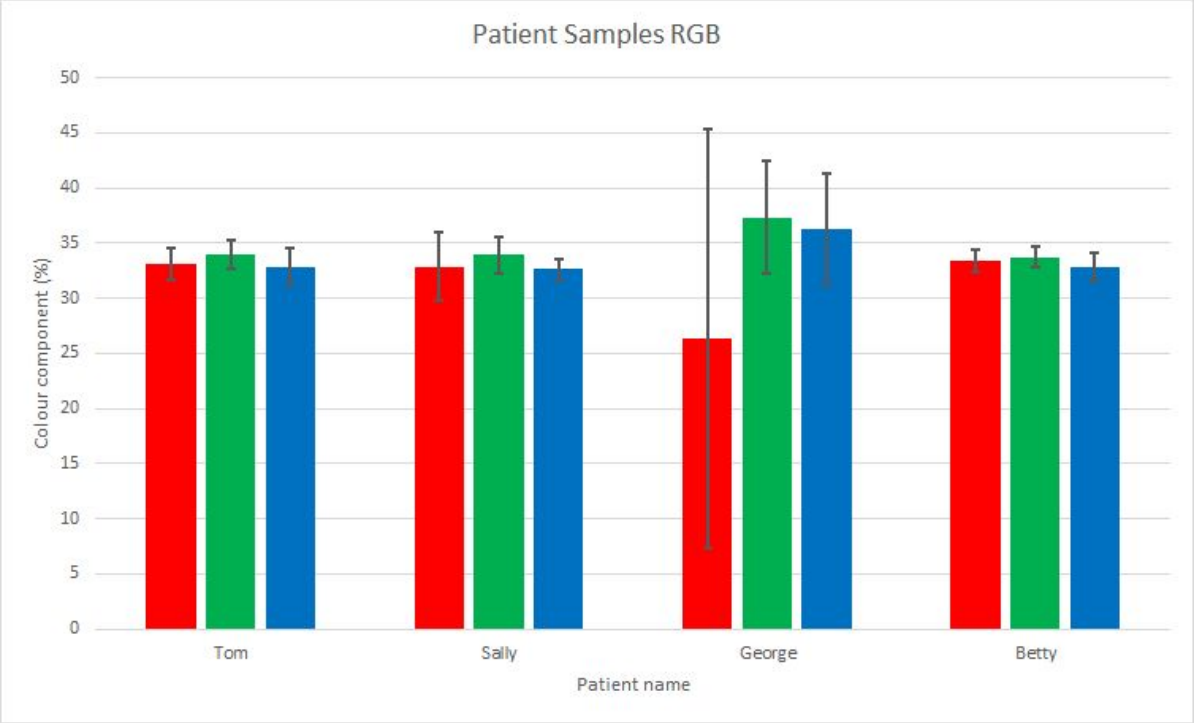


Figure 2 - RGB Values of Patient Samples

A graphed relationship between the green percentage and concentration of the solution is present in *figures 4* and *5* in **4.3 Discrepancy of Results**. Calculations for the concentrations of glucose within the patient samples are performed and tabulated in Appendix I and J respectively.

3.2 Multistix® Patient Samples Results

Sensi-GO™ is a quantitative sensor that reads the RGB value of the Sensi-GO™ reaction as an indicator of the presence of glucose. Multistix® is a qualitative sensor that uses a colour indicator test strip and a colour chart (Appendix H) to get an approximate associated value. This was used to complement the patient sample results (refer to *figure 3* for Multistix® results).

Figure 3 - Photo of Patient Samples Using Multistix®

The column of interest is the second column from the left for glucose readings. These results will be discussed in-depth in **4.4 Sensi-GO™ Detection**.

4. Discussion

4.1 Sensitivity and Specificity

The sensitivity and specificity of the Sensi-GO™ technology can be determined. The counts of positive and negative results are tabulated in *table 4*:

Table 4 - Count of Results of Patient Samples

	Positive Result Count	Negative Result Count	Total
Glucose Present	1	0	1
Glucose Non-Present	0	3	3
Total	1	3	4

The sensitivity formula [6] is: $$Sn = \frac{True\ Positives}{True\ Positives + False\ Negatives}$$

$$Sn = \frac{1}{1+0} = 100\%$$

The specificity formula is: $$Sp = \frac{True\ Negatives}{True\ Negatives + False\ Positives}$$

$$Sp = \frac{3}{3+0} = 100\%$$

The Sensi-GO™ testing method has a 100% success rate in both sensitivity and specificity as there are no false positives or false negatives present. However, due to the lack of test samples and the limited number of trials, this should not be definite.

4.2 Design of Diagnostic Test

The medium to handle the sample solutions for measurement was standard filter paper cut into 50mm x 5mm strips. The selection of medium was influenced by its advantages and disadvantages in *table 5*.

Table 5 - Advantages & Disadvantages of Filter Paper

Advantages	Disadvantages
Small size concentrates colour into easily measurable area	Sample solutions and Sensi-GO™ solutions is not homogenous
Filter Paper is inexpensive and readily available	Solution bleeds through paper over time
Provides a white backing to show true colour	-

The filter paper was chosen primarily on its inexpensiveness, availability, and appropriate small size and neutral colour. Due to small sample sizes, it was determined that discrepancies from the homogeneity of sample solutions were negligible. The bleeding of solution through filter paper was negligible due to the relatively short experimental time.

4.3 Discrepancy of Results

The percentage of green in each glucose and sucrose trial was plotted with error bars and line of best fit.

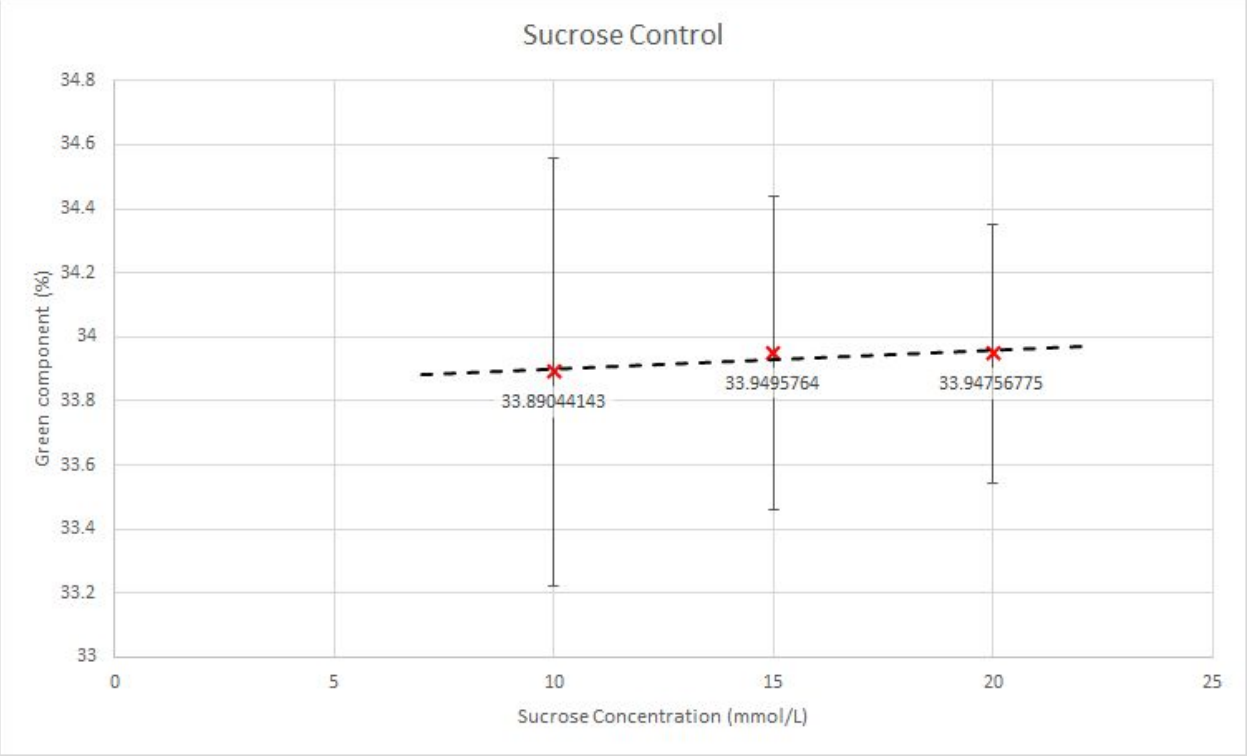


Figure 4 - Green Percentage in Sucrose Solutions

From *figure 4*, the line of best fit is:

$$y_S = 0.0057 \times x + 33.844$$

Where y_S is the percentage of green of sucrose and x is the concentration of sucrose. This trendline is close to a horizontal line as expected as sucrose trials act as the control variable to the clinical trial. However there is a large standard deviation of 0.7%, 0.5%, and 0.3% for 10, 15 and 20 mM of sucrose which indicates the unreliability of sucrose solution results and impreciseness to the true value of the percentage of green.

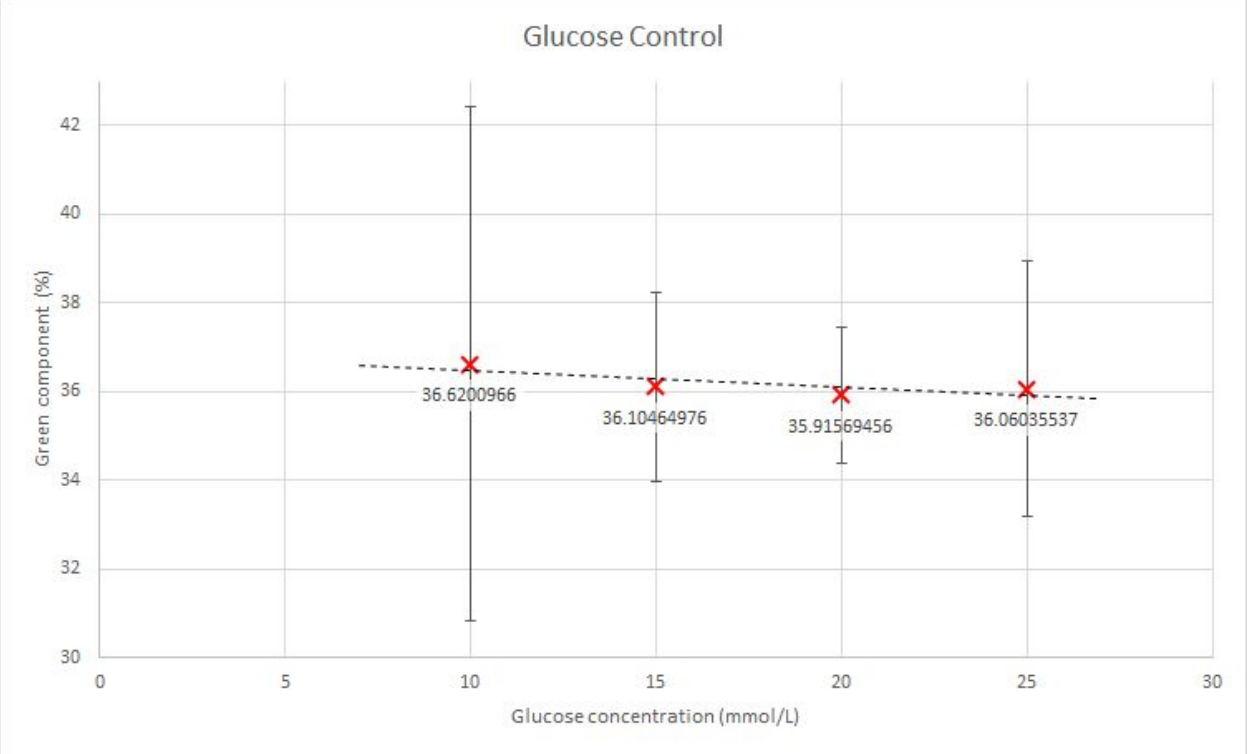


Figure 5 - Green Percentage in Glucose Solutions

From *figure 5*, the line of best fit is:

$$y_G = -0.0374 \times x + 36.829$$

Where y_G is the percentage of green of glucose and x is the concentration of glucose. This trendline did not conform to the expected positive linear trend. Therefore the relationship between the glucose concentration and percentage of green is invalid.

Sucrose and glucose results are extremely unreliable and imprecise with large discrepancies in each trial. Discrepancies may be due to:

- distance and angle between the camera and subject
- small volume of solution
- different pipetted volumes of solution
- heterogeneous solution and colour due to use of filter paper
- light interference due to room lighting and sunlight

The measurements were taken without a fixed distance and angle between the camera and subject. This would cause some discrepancy as 'Color Grab' is very sensitive.

3mL pipettes were used to transfer all solutions. Small volume transfers have systematically larger errors in the number of moles of reactants. Therefore the concentration of glucose will vary with each trial - affecting the concentration of ABTSG. In addition, there was not a consistent pipetted volume onto the test strip.

The use of filter paper in this case did not allow the homogenous mixing of the sample and test solutions. Therefore sample solutions after Sensi-GO™ reactions were not uniform in colour. This was a large contributor to the discrepancy in the percentages of green of glucose solutions.

4.4 Sensi-GO™ Detection

From *table 3* of Appendix F Tom, Sally, and Betty had similar percentages of green component (33.99%, 33.91%, 33.76%) whereas George had a significantly greater percentage (37.32%). This was confirmed by Multistix®. With reference to *figure 3* for patient sample results obtained using Multistix® and Appendix H for the Multistix® colour chart - Tom, Sally and Betty had a negative glucose reading. George had a deep brown colour indicating glucose concentrations greater than $2000\ mg/dL$ within his urine sample. Where the glucose concentration, using the Joslin Diabetes Center's conversion chart [2], in millimolars is:

$$2000\ mg/dL\ =\ 2000 \times 0.06\ mMol/L = 120\ mM$$

The differences in results between Sensi-GO™ and Multistix® are due to invalid Sensi-GO™ results (see Appendix J) extrapolated from *figure 5*, therefore quantitative measurements cannot be made about the concentration of glucose.

George's Sensi-GO™ and Multistix® glucose readings combined with his patient information from Appendix A; George's age (32) and other symptoms, *"Frequently thirsty and produces a large volume of urine (4L per day). He is mildly overweight and lethargic,"* are strong signs of diabetes.

4.5 Effect of Heat and Oxygen Levels on Sensi-GO™ Results

An enzyme's optimal working temperature is $50^{\circ}C$ for glucose oxidase [3] and $30^{\circ}C$ for horseradish peroxidase [4] (refer to Appendix D and E for enzyme activity vs temperature graphs). Low temperatures decrease the kinetic activity of the enzyme due to a loss in the active site's flexibility. A high temperature denatures the enzyme and destroys the protein. The experiment was conducted during the 1/07/2019, where a maximum temperature of $19^{\circ}C$ was recorded [5], meaning that the experiment saw decreased enzyme kinetic activity. This means the intensity of green produced may have been limited.

Since the sample solution was not mixed for homogeneity, insufficient oxygen levels for Sensi-GO™ reactions (Appendix B) may have oxygen become the limiting reagent to the production of glucose, decreasing the production of ABTSG.

4.6 Sources of False Negatives

A false negative in this clinical trial experiment would indicate a patient to not have diabetes when they actually do have it. Despite having a sensitivity and specificity of 100% from **4.1 Sensitivity and Specificity**, possible sources of false negatives are:

- ➢ effects of heat and oxygen on Sensi-GO™ results;
- ➢ and excess intake of water.

As discussed in **4.5 Effect of Heat and Oxygen Levels on Sensi-Go Results**, oxygen acting as the limiting reagent and any temperature from the optimal enzyme working temperature will cause a decrease in the production of ABTSG therefore causing a false negative.

An excess intake of water would cause urine samples to become more diluted therefore lowering the glucose concentration which would cause a false negative reading.

4.7 Sensi-GO™ Test

The Sensi-GO™ test did not pass the clinical trial due to extremely unreliable results as discussed in **4.3 Discrepancy of Results**. There are no means to accurately measure the concentration of glucose in the patient sample using the Sensi-GO™ method.

4.8 Improvements

The issue of lack of controlled variables must be addressed. The first controlled variable would be the process of recording the colours from the samples; variations in angle could be addressed by fixing the mobile phone in place. The second controlled variable is the lighting of the room. The experiment should be performed in a uniformly lit environment such as a purpose-built lab. This is because the mobile phone camera is very sensitive, so local disturbances will influence the results. The third controlled variable is the use of more precise pipettes, as the plastic ones used in the experiment were prone to dropping twice the intended amount of substance, potentially skewing the results.

5. Conclusion

The clinical trial experiment aimed to determine the concentration of glucose in four patient urine samples. Sensi-GO™ was complemented with Multistix® results which both confirmed George to have a high presence of glucose in his sample. Combined with his medical history, he is a strong candidate to have diabetes. However Sensi-GO™ and Multistix® could not be compared quantitatively due to invalid Sensi-GO™ results from the measurement of various glucose concentrations which arose from the lack of controlled variables and discrepancies such as the distance and angle between camera and subject, use of filter paper leading to nonhomogeneous solution, interference of light, error in the volume transfer of a small 3mL pipette, and effects of heat and oxygen levels. Therefore Sensi-GO™ has failed the clinical trial experiment and further revisions to experimental procedure is advised.

6. References

[1] AIHW (2018). How many Australians have diabetes?. https://www.aihw.gov.au/reports/diabetes/diabetes-snapshot/contents/how-many-australians-have-diabetes [Accessed 14/07/2019]

[2] Joslin Diabetes Center. Conversion Table for Blood Glucose Monitoring. https://www.joslin.org/info/conversion_table_for_blood_glucose_monitoring.html [Accessed 28/07/2019]

[3] Belyad, F., Karkhanei, A. A., Raheb, J. (2018). Expression, Characterization and One step Purification of Heterologous Glucose oxidase Gene from Aspergillus niger ATCC 9029 in Pichia pastoris. https://www.researchgate.net/publication/327407747_Expression_Characterization_and_One_step_Purification_of_Heterologous_Glucose_oxidase_Gene_from_Aspergillus_niger_ATCC_9029_in_Pichia_pastoris#pf4 [Accessed 20/07/2019]

[4] Mohamed, S. A., Darwish, A. A., El-Shishtawy, R. M. (2013). Immobilization of horseradish peroxidase on activated wool. https://www.sciencedirect.com/science/article/pii/S1359511313001049 [Accessed 20/07/2019]

[5] AccuWeather (2019). https://www.accuweather.com/en/au/sydney/22889/july-weather/22889 [Accessed 20/07/2019]

[6] Parikh, R., Mathai, A., Parikh, S., Sekhar, G. C., Thomas R. (2008). Understanding and using sensitivity, specificity and predictive values. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2636062/ [Accessed 28/07/2019]

7. Appendix

Appendix A - Patient Sample Information

Name	Age	Symptoms
Tom Jones	20	Produces a small volume of urine frequently and complains of discomfort during urination. No evidence of urethral discharge while microscopic examination reveals a small number of red and white blood cells.
George Murray	32	Frequently thirsty and produces a large volume of urine (4L per day). He is mildly overweight and lethargic.
Sally Smith	28	Patient suspects that she may be pregnant and has visited the doctor to have a pregnancy test (measures human chorionic gonadotropin (HGC) levels).
Betty Crocker	65	Joint pain and an unusual rash.

Appendix B - Sensi-Go Chemical Reactions

$$C_6H_{12}O_6 + H_2O + O_2 + GOx \rightarrow C_6H_{12}O_7 + H_2O_2 + GOx \quad (1)$$

$$H_2O_2 + ABTS + HRP \rightarrow H_2O + ABTSG + HRP \quad (2)$$

$GOx = Glucose\ Oxidase\ Enzyme$

$ABTS = Colourless\ Peroxidase\ Substrate$

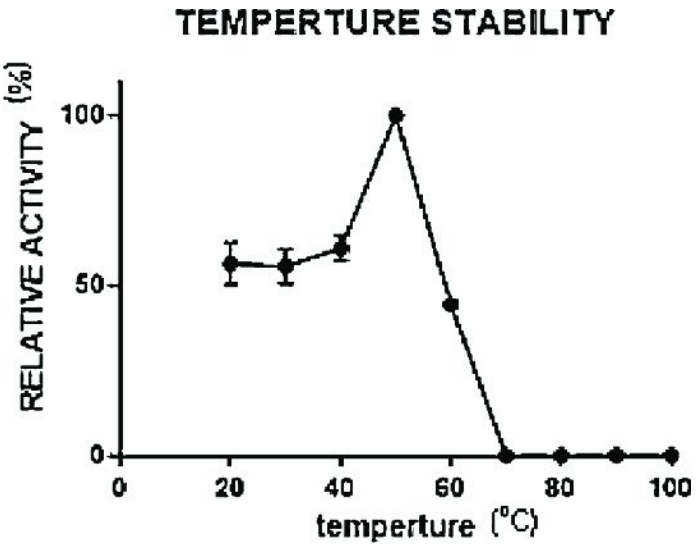
$ABTSG = Dark\ Green\ Coloured\ Peroxidase\ Substrate$

$HRP = Horseradish\ Peroxidase$

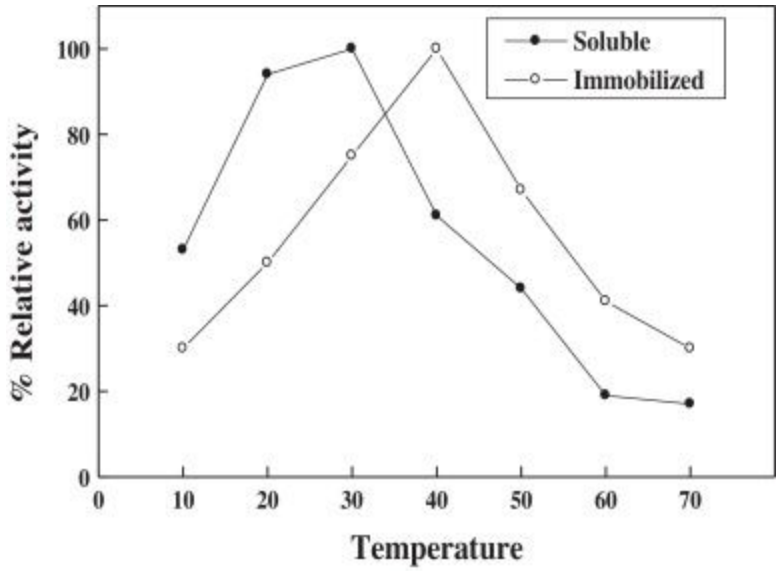
Appendix C - Clinical Trial Laboratory Experimental Procedure

1. Cut at least 33 50mm x 5mm test strips from filter paper.
2. Pipette one drop of 10 mM glucose solution onto 3 different test strips. These will be used as standards for positive results.
3. Repeat step 2 for 15, 20, 25 mM and identify each strip with a marker by its concentration and solution type.
4. Pipette 1 drop of part A Sensi-GO™ onto the test strips.
5. Pipette 1 drop of part B Sensi-GO™ onto the test strips.
6. Get the RGB value of the solutions on each test strip using the Colour Grab application.
7. Record the RGB values in a table
8. Pipette one drop of 0 mM sucrose solution onto 3 different test strips. These will be used as standards for negative results.
9. Repeat steps 4-8 for 10, 20 mM and identify each strip with a marker by its concentration and solution type.
10. Pipette one drop of a patient's samples onto 3 different test strips.
11. Repeat steps 4-8 for each patient sample.

Appendix D - Kinetic Activity of Glucose Oxidase Enzyme vs Temperature



Appendix E - Kinetic Activity of Horseradish Peroxidase Enzyme vs Temperature



Appendix F - Tables of Results

Table 1 - RGB of Glucose Solutions

		Glucose Solution (mM)			
		10	15	20	25
RGB of Trials	1	142, 185, 180	151, 189, 185	170, 200, 195	172, 198, 193
	2	118, 174, 174	157, 192, 187	161, 192, 184	146, 190, 189
	3	148, 189, 185	140, 186, 183	138, 185, 180	144, 185,170
Average RGB		136, 183, 180	149, 189, 185	156, 192, 186	154, 191, 184
Average RGB % Component		27.37 36.62 36.01	28.56 36.10 35.34	29.29 35.92 34.80	29.16 36.06 34.78

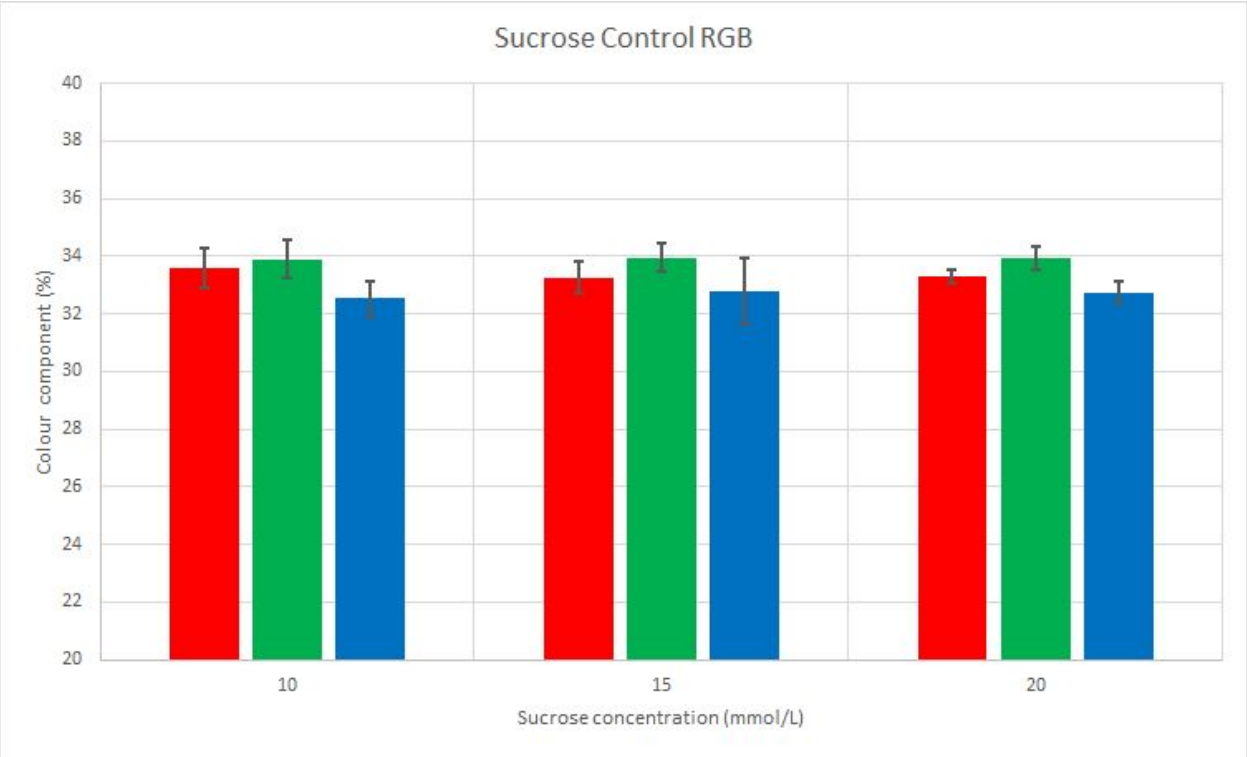
Table 2 - RGB of Sucrose Solutions

		Sucrose Solution (mM)		
		0	10	20
RGB of Trials	1	216, 218, 208	208, 213, 208	212, 217, 209
	2	211, 213, 205	208, 212, 201	210, 214, 207
	3	216, 218, 210	212, 216, 210	211, 214, 206
Average RGB		214, 216, 208	209, 214, 206	211, 215, 207
Average RGB % Component		33.58 33.89 32.53	33.26 33.95 32.79	33.32 33.95 32.74

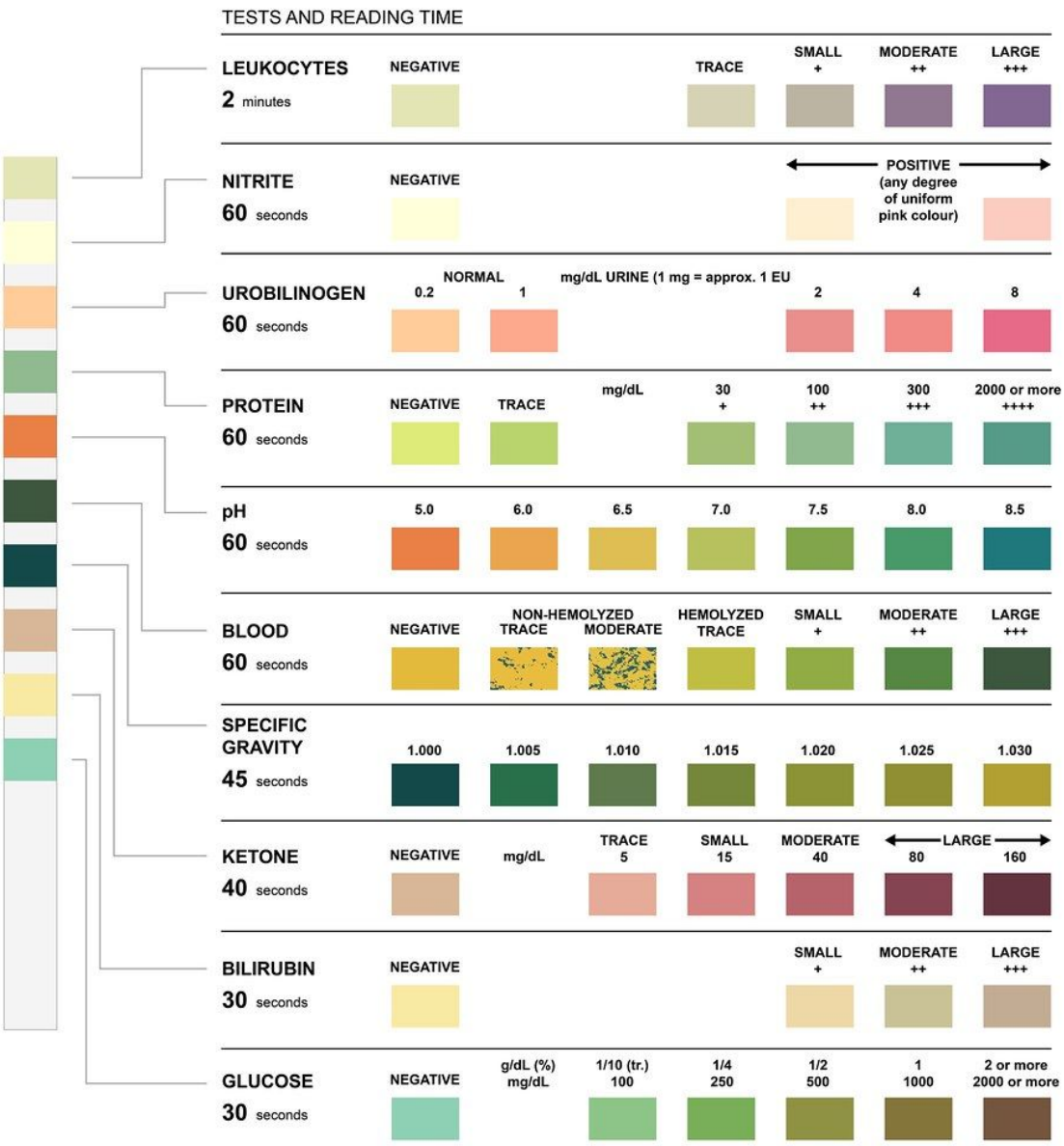
Table 3 - RGB of Patient Samples

		Patient Samples			
		Tom	Sally	George	Betty
RGB of Trials	1	211, 216, 210	224, 223, 213	106, 169, 169	220, 218, 211
	2	207, 212, 204	211, 216, 209	149, 185, 179	217, 222, 215
	3	205, 211, 204	215, 220, 212	111, 170, 162	216, 220, 216
Average RGB		208, 213, 206	217, 220, 211	122, 175, 170	218, 220, 214
Average RGB % Component		33.14 33.99 32.87	32.90 33.91 32.63	26.36 37.32 36.32	33.40 33.76 32.84

Appendix G - RGB of Sucrose Solutions



Appendix H - Multistix® Colour Chart



Appendix I - Example Calculation for the Glucose Concentration Using George's Sample

Using the glucose line of best fit:

$$y_G = -0.0374 \times x + 36.829$$

$$37.32 = -0.0374 \times x + 36.829$$

$$\therefore x = -13.1\ mM$$

Appendix J - Table of Glucose Concentrations of Patient Samples

	Patient Samples			
	Tom	Sally	George	Betty
Glucose Concentration (mM)	75.9	78.0	-13.1	82.1

These results extremely deviate from the reasonable assumption that an increase in the percentage of green component would have a linear increase in glucose concentration. Therefore these results are invalid and will not be used in discussion.