Optimized Colorimetric Test Strips & Mobile App Accompaniment for Blood Glucose Regulation

Catherine Chen, Vincent Wang, Peter Baccarrella,
Jonathan Lam, Amy Leong, and Emily Yasharpour

The Cooper Union
for the Advancement of Science and Art

EID-101 Section E
Professor Anita Raja
December 20th, 2018

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Diabetes mellitus (diabetes) is the group of diseases characterized by excessive blood glucose levels due to the body's inability to produce or use insulin. Regular blood sugar tests are required for the well-being of diabetics, but in many developing nations there are no means to inexpensively and effectively measure blood glucose levels— the most prevalent method is to use an electrochemical glucometer with one-time-use test strips. Glucometers typically range from roughly $25 to $60 and individual test strips cost between $0.40 to $2.00. This financial constraint is dire in countries such as Uganda, where the average income in Kampala, its largest city and second wealthiest district by GDP per capita, is roughly $300 per month (UBOS, 2017).

Our project uses indicator solutions to visually determine blood glucose levels by a color change, extending the research from the 2017 EID-101 Section E G-Cubed group. Glucose oxidase, the primary reagent for glucose, and TMB, the primary indicator, create the color-changing chemical reaction. The solution will be distributed in bottles to be dispensed dropwise onto copy paper. A fibermesh membrane will be placed on top of the paper to filter out red blood cells. A mobile application that uses camera input to reasonably estimate the glucose level based on the color of the strips was also created. A study by the Uganda Bureau of Statistics found that 86% of 18 to 30 year-olds own a smartphone and most Kampala households have access to a smartphone so a mobile app is reasonable (UBOS, 2017).

The product would be distributed in a kit with a bottle of of glucose oxidase-TMB solution and the fibermesh membrane, as well as instructional manuals and a link to the app. A cost analysis showed that each strip averaged out to less than one cent per unit, meeting the low-cost design criteria.

DESIGN PROBLEMS AND OBJECTIVES

The 20th century marks the rise of the diabetes pandemic. The 21st century marks the rise of the diabetes epidemic. With 1.6 million deaths and drastic yearly increases in diabetic prevalence, diabetes is on track to becoming the leading cause of death of South Africa by 2040. The situation is especially serious in Sub-Saharan Africa, where rapid urbanization and changes to a western lifestyle and diet only further increases the diabetic population. This project will target Kampala, Uganda, one of the fastest growing cities in the world (Vermeiren, 2012). Here, the diabetic population is estimated to projected to increase 4.2% annually (UBOS, 2017).

To ensure the diabetic's well-being, blood glucose levels have to be regulated daily. Over the span of a single day, external factors such as food consumption cause blood glucose levels to fluctuate. In order to properly use insulin, an accurate and recent reading of the diabetic's blood glucose level is necessary. However, the combination of financial costs of glucose regulation and lack of cultural acceptance for diabetics in Kampala make regulation extremely difficult.

Therefore, this project focused on optimizing a low-cost and sustainable glucose management device for the diabetics in Kampala, Uganda based on the results of the 2017 EID-E group. The other design criteria were ease of use, safety, and cultural sensitivity. Since the 2017 EID-E group had achieved a low-cost colorimetric test strip by reducing its function to simply measuring blood glucose levels, our colorimetric strip aimed to lower the cost through different chemical reactions and improve the accuracy of the colorimetric test strip using a red blood cell filtration mesh.

Our main design goal focused around being low-cost. In the United States, diabetics use glucometers and test strips in conjunction to measure their blood glucose level. The cheapest price for a glucometer and test strips are $15 and $0.33 per strip (prices found on Amazon), respectively. Since the average household in Kapala make approximately $287.62 monthly (UBOS, 2012-2013), the test strip was designed to be significantly less than commercial

products through a combination of some sustainable parts and cheap disposable parts. Our design also replaced the necessity of a glucometer with a free mobile applications to further reduce costs. Since Kampala is the capital of Uganda and is in a period of rapid urbanization, this plan is viable because technological literacy is common in the younger generations and phones are ubiquitous in every Kampalan household.

DETAILED DESIGN DOCUMENTATION

I. Solutions Brainstorm and Decision

The problem statement was very broad: to create a low-cost diabetes monitoring device that can reasonably accurately determine blood glucose levels in a safe, secure way. A Duncker diagram and a decision matrix were used to aid brainstorming ideas.

Figure 1a. Duncker Diagram Present State

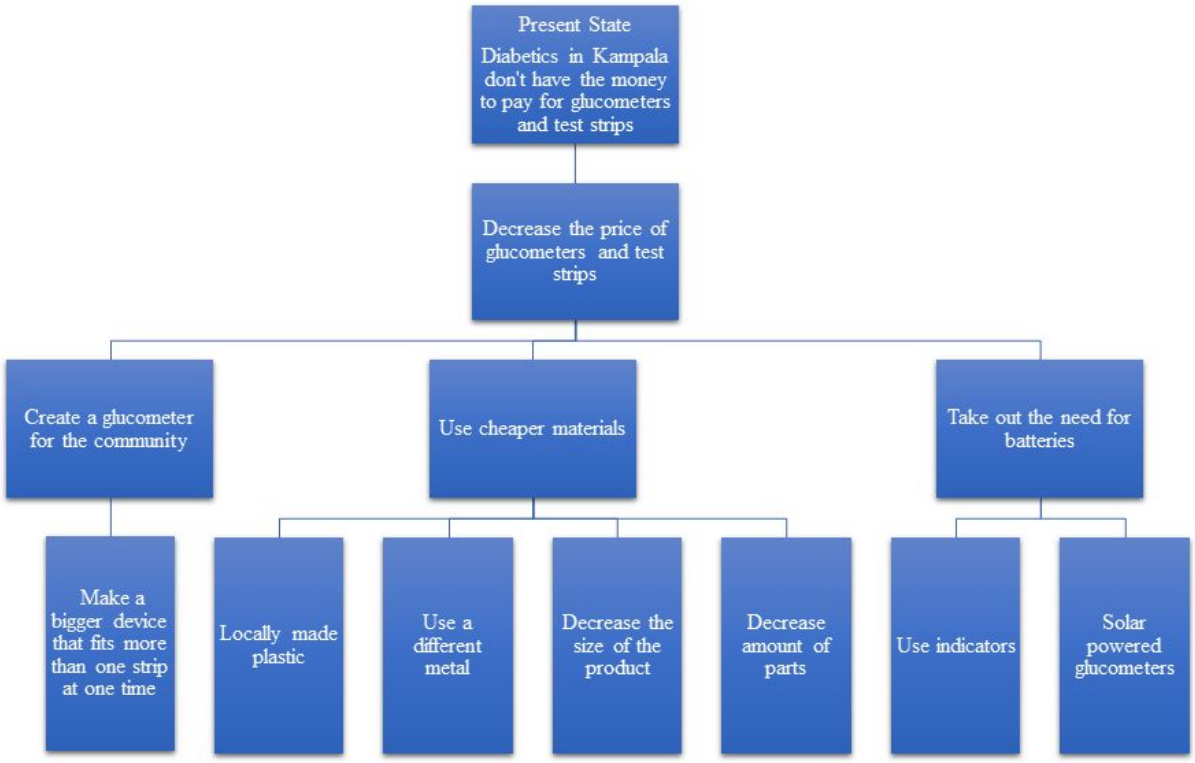
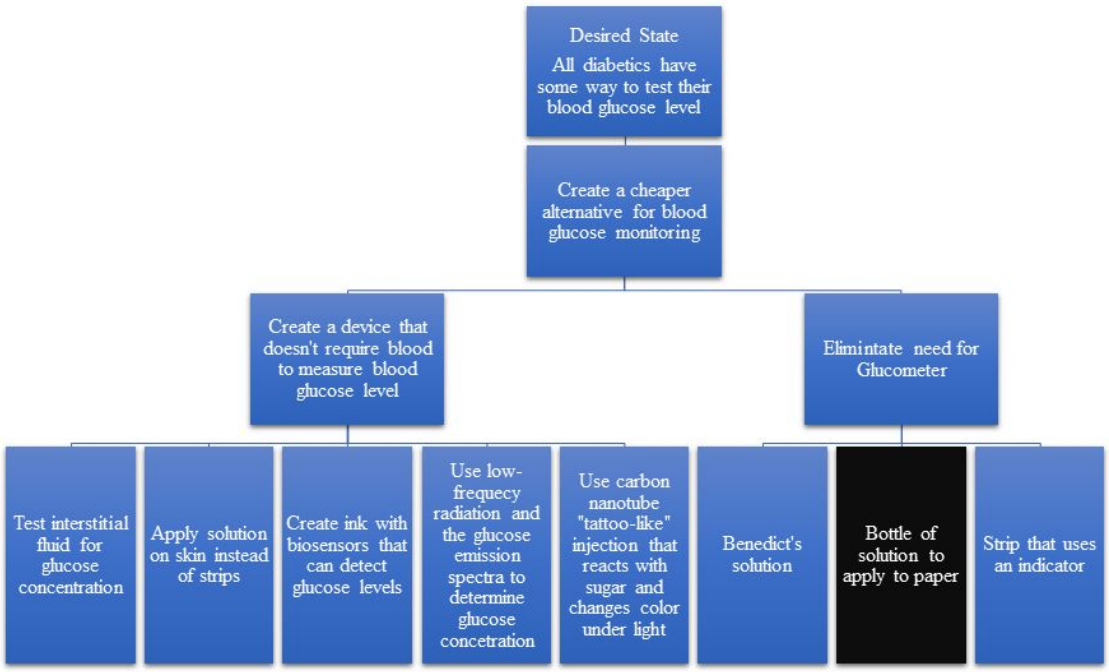


Figure 1b. Duncker Diagram Desired State



A decision matrix was made for some of the top solutions to help determine the best choice. This is shown in Figure 2.

Figure 2. Decision Matrix

	Cost	Portability	Performance	Ease of Use	Sustainability	Locally reproducible	
Weighting	100	50	100	85	60	70	Total
Test strip	9 900	9 450	9 900	7 595	6 360	5 475	3680
Community glucometer	5 500	0 0	9 900	8 680	6 360	4 280	2720
Carbon nanotube tattoo	1 100	9 450	9 900	8 680	8 480	0 0	2610

For the Duncker diagram, there were a few notable solutions on both major branches. If the present state was maintained, then glucometers would still be the main way to test blood glucose, and the project's goal would be to lower the cost of conventional blood glucose monitors to be affordable for Kampalans. The cost of test strips could be decreased if the strips were smaller, which would decrease material costs. It was also suggested to decrease the cost by changing the electrode material away from precious metals like gold into carbon or other materials (test strips originally used carbon, but metal ones proved to be more accurate; however, the accuracy of carbon test strips may be reasonable enough for our project). Lastly, another brainstormed idea was that the test strips could be more effectively sized by tearing it from a roll, so a piece of the test roll as large as it needs to be could be obtained.

However, there was more focus on moving away from traditional electrochemical glucometers and test strips. For these solutions, the technical knowledge of the team is not enough to be able to produce test strips nor glucometers, which are usually manufactured in factories.

For the decision matrix, various design alternatives were considered so that the strengths and weaknesses could be taken into account. It allows us to review each alternative thoughtfully by prioritizing our goals. The goals for this project consisted of cost, portability, performance, ease of use, sustainability, and locally reproducible. Since we mainly wanted to focus on the low cost and the performance aspect, we decided that those factors would weigh the most. We wanted to create something that the people of Kampala can buy at a reasonable price, while it being as accurate as possible. This sets the test strip at an advantage already since it is the most inexpensive method. However, the test strips were ranked as one of the lowest in terms of ease of use and sustainability. Since those factors were not ranked as high, it was a compromise that was worth taking. With the decision matrix, we were able to narrow down some alternatives and were able to see that the color test strips was the best method as the total is the highest with the score of 3680

Additionally, existing research on glucose monitoring devices not involving glucometers mostly were non-invasive solutions, or did not require drawing blood samples. For example, a study was conducted at the University of Bath about the potential for an adhesive patch that measured the glucose concentrations of interstitial fluid ("Bloodless Revolution in Diabetes Management," 2018). A study by MIT studied the potential for a carbon nanotube tattoo comprised of materials that appear different colors, depending on blood glucose concentration, under near-infrared light (Jensen 2011). Solutions such as these were very interesting, but the equipment, materials, and skills necessary would be far beyond the scope of the team's expertise. It was also believed that these types of solutions would be too costly to fit the design criteria, and would mostly be used in first-world countries where drawing blood may be a problem.

The last source was that of a team in the EID-101 Section E class of 2017 (Chan et al, 2017). They created a "colorimetric test strip," which used an indicator to test the products of a glucose oxidation reaction. This allows the user to visually determine blood glucose level based on color, without any equipment. While this still requires blood drawing like a glucometer, it is purely chemical (not requiring an electrical power source), and the analysis is optical (not absolutely requiring any special equipment for a sighted person). The group developed an app to work alongside it to ease the blood glucose level (BGL) determination. Interestingly, they had tried to use an indicator, TMB, known to change color with glucose, which failed; they turned to the commonplace starch-iodine colorimetric reaction, which appeared to perform well.

This final design was chosen because it was promising, the level of technicality was within the reach of the team, and still had many aspects to improve upon. Because their idea was novel, the 2017 research team encountered and listed many problems in their report, such as:

1. The TMB changed color prematurely, probably due to improper use.
2. Adding an colorimetric indicator to a solution containing blood was contaminated with the red color of the red blood cells (RBCs).
3. As a drop of the indicator dried, it formed rings and gradients of different colors.

4. The mobile app was not complete, perhaps mostly due to time constraints. The color analysis was a simple averaging of colors, which was not ideal due to problem 3. There was little data used to calibrate the app, and no report was given to the accuracy of its BGL determination.
5. Lighting was inconsistent. An attempt was made to make lighting consistent by using using the app in a box, but there is little data about how well this worked.

The 2017 research team informed us of these issues, and there was regular communication between their team and ours. Our design focused on tackling each one of these problems. A more specific breakdown of our team's goals can thus be written analogously to these problems:

1. Correctly use the TMB indicator, and figure out the conditions of best use.
2. Filter out red blood cells before use with indicator.
3. Find a way to analyze the image differently to account for the changes in rings. For visual BGL determination, choose a metric that is easy for people to understand that takes this into account.
4. Take more time to complete a mobile app. Run a greater number of trials in a wide variation of conditions, and estimate its accuracy.
5. Take lighting into account when measuring the blood glucose level, especially in the app's algorithm.

To tackle this wide variety of problems, the group was divided into multiple subteams. The subteam and role of each person is displayed in Table 1.

Table 1. Team Member Roles

Name	Team	Roles
Peter Baccarella	Chemistry	Laboratory worker
Catherine Chen		Laboratory worker, production of mesh
Vincent Wang		Laboratory worker
Emily Yasharpour		Laboratory worker, production of video
Jonathan Lam	Technology	Webmaster, software developer
Amy Leong		Algorithm development, notetaker

A Gantt chart of the project schedule is displayed in Figure 3. The timeline for the Gantt chart begins roughly when the solution idea was finalized.

Figure 3. Gantt Chart

	Week									
Task	10/8	10/15	10/22	10/29	11/5	11/12	11/19	11/26	12/10	12/17
Preparation										
Brainstorm and Project Proposal										
Finalize chemicals										
Research and Development										
Order materials										
Collect data and test chemicals										
Mesh R&D										
Software										

development										
Final Deliverables										
Website development										
Technical report										
Video editing										

Unfortunately, a large part of the research and development phase began very late, so that much of the data collection occurred in the last two to three weeks. This delayed some of the progress on the mobile app development and addition of content to the website.

II. Chemical Design

The idea of a colorimetric test strip was tested by G-Cubed solutions (Chan et al, 2017) the previous year. The test strip currently consists of a mixture of chemical indicators, a filter mesh and a small strip of plain white printer paper. The printer paper was chosen because it is the cheapest, uniform paper that was found. The chemical mix is made up of TMB or 3,3',5,5'-Tetramethylbenzidine, Horseradish peroxidase, and glucose oxidase dissolved in a mixture of ethanol and deionized water. When it comes into contact with glucose, the glucose oxidase will break it down and produce gluconic acid and hydrogen peroxide. Horseradish peroxidase then oxidizes the TMB using hydrogen peroxide and produces a blue color change. The filter mesh is used to filter out the Red blood cells from the sample of blood. This is done to remove the red color in the blood as to not affect the color of the test. The filter mesh is made up of electrospun polymers with a weave small enough to limit the passage of cells including RBC through it.

The mixture of chemicals and the amount to put on the test strip was found based on the effective units of each reagent. The Glucose oxidase mixture was 30 units per ml, the Horseradish Peroxidase was 60 units per ml and it was decided to make the TMB mass equal to the peroxidase due to a lack of unit measurement.

The test strip is made by placing the sample blood through the filter mesh, onto the piece of paper then adding the chemical mixture. This was found to be the best order due to the red color that developed from putting the chemical mixture first.

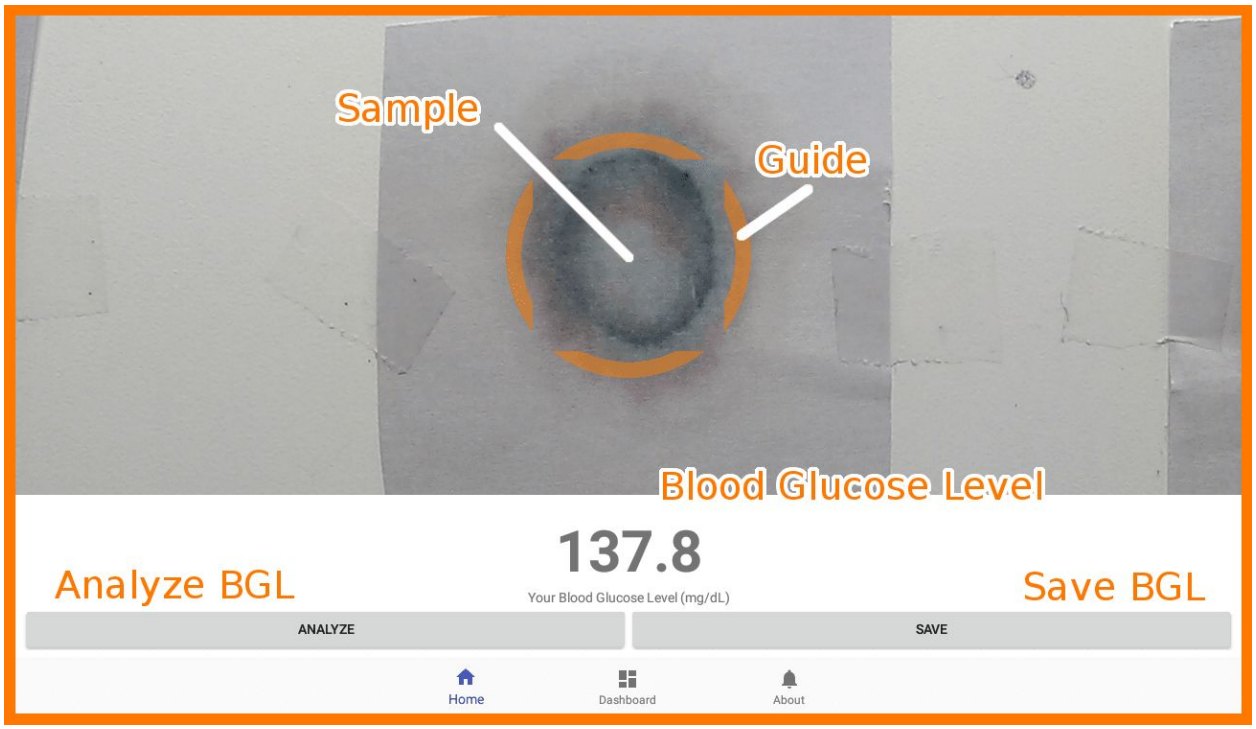
III. App Design and Color Analysis

The idea of using a mobile device (with a camera input) as a method to more accurately determine blood glucose level from the test strips optically was initially tested by G-Cubed Solutions (Chan et al, 2017). While it may seem unreasonable to restrict app access to those users who have smartphones, the Uganda Bureau of Statistics (UBOS) found that 86% of Kampalans from ages 18 to 30 own a smartphone, and that most Kampala households have access to a smartphone (UBOS, 2017). There is no cost to obtaining a (free) mobile application from a mobile application vendor (e.g., iOS's App Store or Android's Play Store), with only the one-time need for Internet access for its download. The prevalence of capable computing devices in Kampala, as well as the absence of cost of distribution, make the idea of mobile-application identification capable for reaching a large part of the target population.

The mobile app has three tabs. The first would be for scanning an image to get blood glucose level; this comprises of a live camera stream (see Figure 4), with markers to guide the user where to place the colored spot on the paper; this was the most important part of the app, and the most significant time was placed in this area. The second and third tabs would be a tabulated list of

blood glucose levels from past scans along with timestamps of the measurements, and the final tab is a simple how-to page. The blood glucose level determination in the app works in two stages: color detection and color analysis.

Figure 4. Screenshot of Tab to Scan Image and Determine BGL



The color detection by the G-Cubed group asked users to select the region of the image with the color from the indicator solution, and the color in that region was averaged. The color was then approximating using a HSL linearization (hue, saturation, lightness) color values on a model generated by two test data points at a low and high BGL. The initial algorithm for The Regulators involved thresholding out “background” pixels and averaging the rest of the pixels; however, this was not effective for images with uneven background colors or indicator colors with multiple rings, as the samples had. A more efficient method involving clustering similarly colored pixels was used. Contrary to the G-Cubed solution, RGB values were used; HSL was only used in the very early experimentation stages.

Stage 1: The current color detection is a set of heuristics determined somewhat by trial and error. The major steps were threshold all of the pixels into clusters (i.e., breaking down the 2^{24} color space down to 2^{12} clusters), and filtering out clusters based on number of pixels (too few would indicate an insignificant splotch, such as a speck of dust, and too large might indicate a background color), center (the center of the clustered pixels should be near the center of the camera input), error using the "jump method" (Ercolanelli, 2016), and color (the dark blue ring was determined to be the best indicator of color, and thus the algorithm biased dark and primarily-blue clusters). The few clusters that remained would be averaged (weighted averaged based on number of pixels per cluster), and the R, G, and B values of this averaged cluster would be determined to be the most useful ring for the blood glucose level determination.

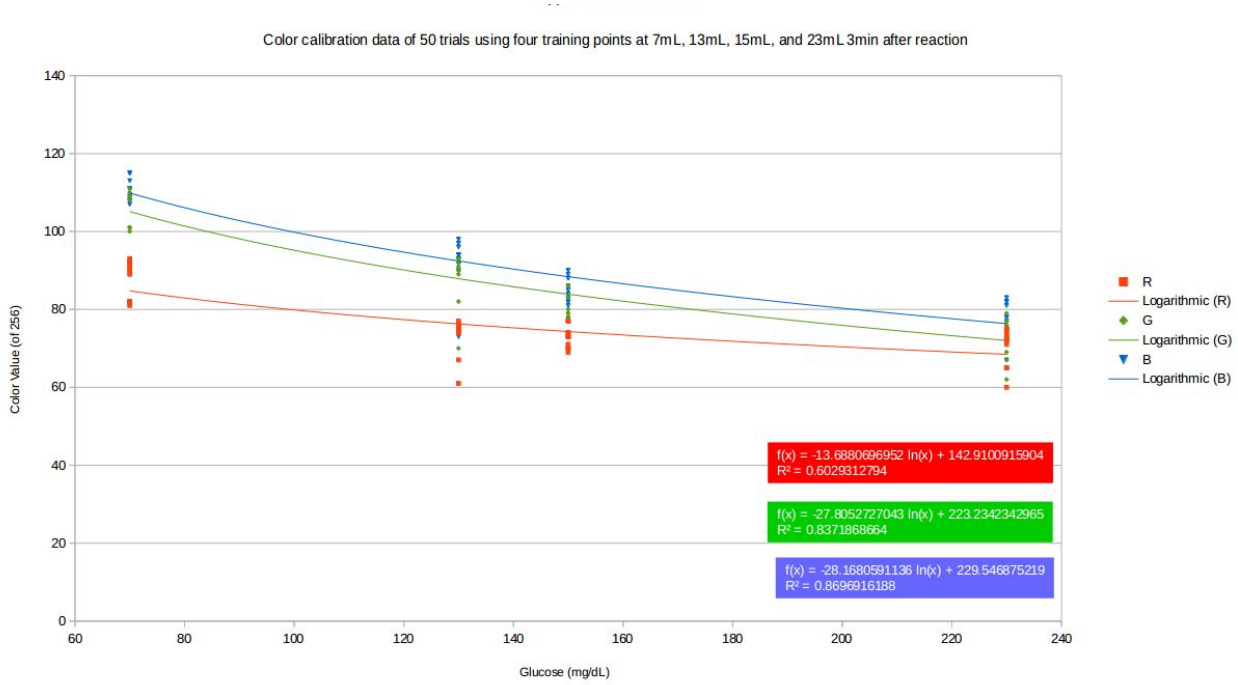
Unfortunately, the color thresholding is slow (it cycles through every pixel, and the time necessary to analyze one scan is roughly 10 to 20 seconds); there were no attempts to improve performance because this is only a minor inconvenience, and this will be an area for further study with potential for downsampling or smarter sampling of relevant areas.

Stage 2: The averaged cluster color would be inputted into the inverse trend line equations generated by the model to guess at the blood glucose level. The three estimates (one for R, G, and B) would be averaged (weighted based on coefficient of determination) to get a final BGL determination. This offers results better than the ones from last year's app because it does not require any user input and can capture more complex patterns (i.e., the rings of color).

The calibration "trials" involved running the heuristic filtering on four samples in constant lighting, and plotting the final cluster against BGL concentration. A trend line was created for each color (see Figure 3). (A polynomial trend line worked best, but its end behavior did not make sense; the close-behind logarithmic models seemed more reasonable). Unsurprisingly, the trend line for the blue component was strongest, indicating that the difference between the blue could most reliably be used to determine BGL. For these curves, there was slightly yellow fluorescent lighting as the only light source, and the samples were printouts of images of

laboratory samples for 70mg/dL, 130mg/dL, 150mg/dL, and 230mg/dL BGL samples three minutes after application. These were considered to be a wide range of acceptable BGL levels. The lighting conditions were poor and not varied because of a lack of time for testing, and therefore is an area for improvement in the algorithm.

Figure 5. App Color Calibration Curves (RGB Color Values vs. BGL)



The concentrations of the samples for calibration were known. When the heuristics are performed on these samples, there is some variation. If used correctly and lighting is consistent, the variation in readings of the same sample varied by up to roughly ±30mg/dL. While this may seem like a wide range of error, diabetics' blood sugar levels can range far greater (“Blood Sugar Levels Chart,” 2015), so it should still be a useful metric. With further experimentation, however, it is expected that the the algorithm should improve and variation should decrease, and user error and lighting will be better accounted for.

For Kampalans who lack access to smartphones, an alternative is to use a color guide to indicate blood glucose level from color or appearance. This is a simpler solution; however, as the color is

not even throughout the splotch of color created by the indicator, using this may introduce some user error. This would be similar to the color guide of a universal pH indicator strip, similar to that shown in Figure 6.

Figure 6. Universal pH Indicator Strip with Color Reference

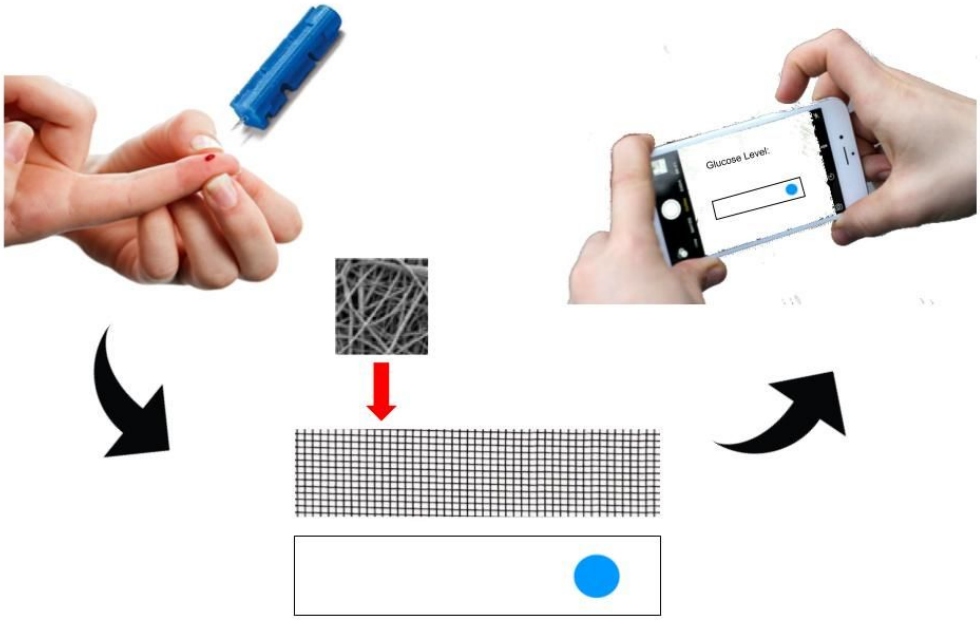
(image courtesy of https://www.grainger.com/product/3UDD2)

Such a color reference has not yet been developed, because more research into what colors (e.g., red, blue green), what aspects of the colors (e.g., hue, saturation, lightness), and what areas or patterns of the image that are most representative of blood glucose level is necessary. Future research into using machine learning with all of the samples conducted will likely create a more accurate color analysis reference. Machine learning should also help account for slight variation in lighting and placement, as well as to potentially speed up image analysis by only analyzing relevant portions of the image.

IV. Overall Product

The final distributable product would be a bottle with a dropper cap of reagent/indicator solution, filter, white paper (optional), instructions to download the mobile application, a color reference guide, a short summary of diabetes and The Regulators project, as well as pictorial how-to manuals for usage of the strip (with filter), mobile application, and color reference guide.

Figure 7. Product Usage



Our product works in three steps. First, the user pricks their finger with a lancet. Next, the user would press their finger against the filtration mesh, which is on top of the test strip. The mesh is removed and a drop of the TMB, horseradish peroxidase, and glucose oxidase solution is placed onto the test strip. After five minutes, there will be a color ranging from pale blue to intense blue. Lastly, the user will take a picture using the mobile app which will give an estimate of the user's blood glucose level.

There were five design criteria: keeping low-cost, sustainable, easy to use, safe, and culturally sensitive.

Tables 2 is a cost analysis of the test solution. The costs are scaled down to costs per mg or mL of solution.

Table 2: Cost Analysis

	Cost per bottle	Cost per mg/mL	Units per bottle
TMB (1mg)	$41.80	$0.04	
GO (1mg)	$45.10	$0.64	10000
HRP (1mg)	$122.59	$0.73	25000
Ethanol (1mL)	$159.90	$0.04	
3mg of TMB/5mL of ethanol	$0.13	$0.21	$0.34
1mg of GO/5mL of deionized water	$0.64		$0.64
3mg of HRP/5mL of deionized water	$2.20		$2.20

In Table 3, the costs from Table 2 are projected to the costs of estimated amounts of the solution per test strip, and this is extrapolated to larger time spans.

Table 3: Projected Costs

	Cost
15ml of test solution	$3.18
1ml of test solution	$0.21
1 strip	$0.001
1 day	$0.003
1 month	$0.098
1 year	$1.159

Table 3 illustrates that a single strip costs less than a cent to make, and that over the span of an entire year, the cost of the solution will be roughly one dollar. There is no additional cost to download the (free) mobile application, provided that the user has access to a mobile phone and a one-time Internet connection. Together, the initial cost $0.01 is much lower than that of a glucometer, and the cost of the solution, at less than a cent per test strip, fits the design goal of less than one cent and is much less expensive than current test strips.

Ease of use is a category that can use some improvement. The product has many components to function properly, and we have not come up with a solution to distribute it as one product, because the solution will degrade when exposed to air and the mesh is reusable. The app is another component that a user has to learn to use. The goal is that with sufficiently thorough instructional manuals, a user should be able to follow the steps efficiently.

Due to the chemicals that we chose, TMB, horseradish peroxidase, and glucose oxidase, it was sustainable and safe to use. If there is any skin contact or eye contact, the chemicals can be thoroughly washed out with water. Also, since there was very little chemicals used per strip, the test strips are sustainable and can be thrown into the trash after use.

When designing our product, we made sure that we conducted enough research about Kampala so that we could understand people different from us and serve their community better. Since there is a lack of medical facilities in Kampala, the wait times are usually long. They would be giving up a day of work in order for them to check the blood glucose levels. This is why we decided to create something that they could use in their own homes instead of travelling to a medical center.

LABORATORY TEST PLANS AND RESULTS

After deciding on the solution we will pursue, a protocol was created so that each step of the process was laid out. At our time in the lab there were three major stages, which were testing in test tubes, testing on paper, and refining our process for testing. This allowed for us to make sure that nothing was wrong with the chemicals themselves and that they were being used correctly before testing them on paper.

First, we needed to make glucose solutions. The concentrations created followed those that a diabetic would experience (DiabetesAdmin, 2015) (See Table 4).

Table 4: Glucose Concentrations

Concentration of Glucose	Standing of blood glucose level
70mg/dl of deionized water	Lower End of Normal for Fasting
130mg/dl of deionized water	Higher End of Normal for Fasting
150ml/dl of deionized water	Lower End of Normal after a meal
230ml/dl of deionized water	Higher End of Normal after a meal

Then, we decided to test the Tetramethylbenzidine(TMB)-Horseradish peroxidase(HRP) reaction in test tubes to make sure the colors presented by the TMB were correct. Concentrations of TMB, HRP, and Glucose Oxidase (GO) were made (Bergmeyer, 1974) (See Table 5).

Table 5: Chemical Concentrations

Chemical	Concentration
Tetramethylbenzidine (TMB)	3mg/5ml of Ethanol
Horseradish Peroxidase (HRP)	3mg/5ml of Deionized water
Glucose Oxidase (GO)	1mg/5ml of Deionized water

We tested the solutions by using the 70mg/dl concentration of glucose and adding each of the other chemicals into the solution in a 1:1:1:1 ratio. The first test turned out to be blue (Figure 8) which is the right color after the reaction but this was found to be due to the fact that the test tube was closed right after the chemical were added. When not closed the color turned out to be orange (See Figure 9).

Figure 8: Solution with cap closed right after all chemicals were added

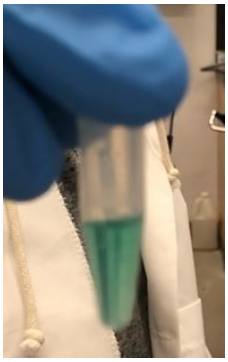
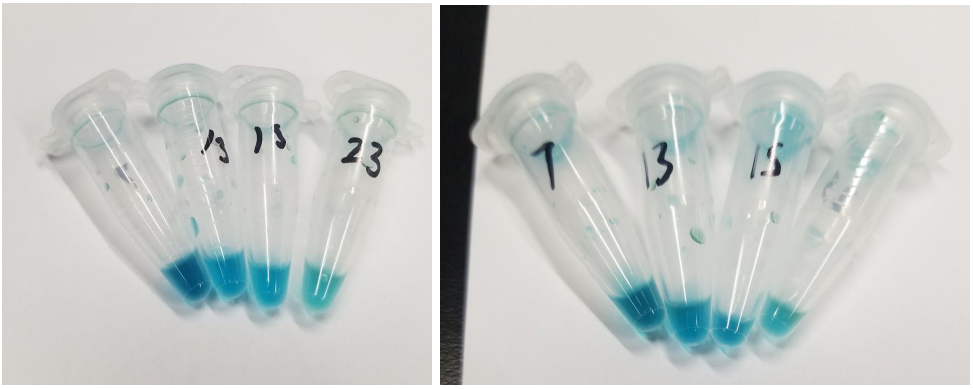


Figure 9: Solution with cap left open

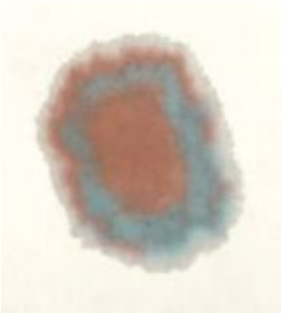
After realizing the effect of leaving the test tube open, we decided to test the color of the solution based on time. Leaving the test tubes open for 5 minutes still gave the solution a blue color (Figure 10) so timing was not a problem as long as it was below 5 minutes.

Figure 10: Solutions for when caps of test tube were closed 5 minutes after chemicals were added: First test (left), Third test (right)



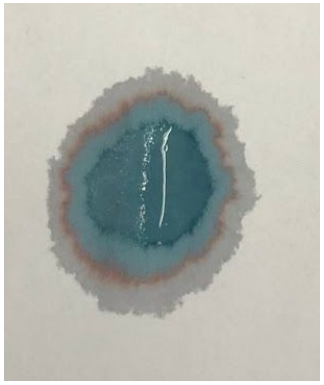
Now knowing that we are using the chemicals correctly, we moved on to creating the test strip. We combined the separate TMB, HRP and GO solutions together in a 1:1:1 ratio to create the test solution which will be put into our product. To create the strip we first added the test solution onto printer paper. Upon the first test, we saw the blue color as expected but what wasn't expected was the red color because the tests in the test tube never showed a red color (Figure 11).

Figure 11: First test with TMB with TMB on the paper first



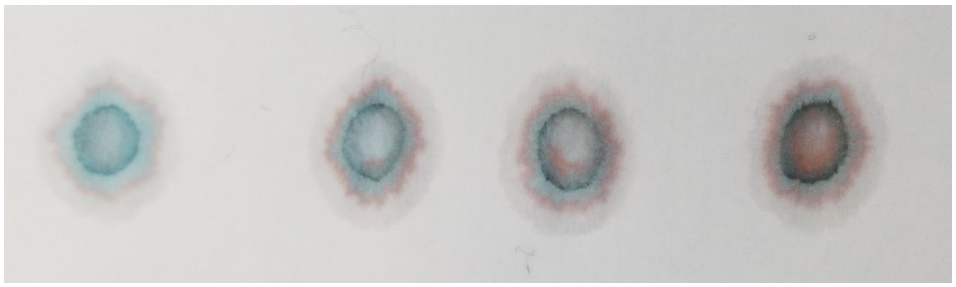
To decrease the amount of red in the color we decided to compare testing with TMB on the paper first (Figure 11) and glucose on the paper first (Figure 12) and we concluded that the best outcome was from the glucose on the paper first because it had the most amount of blue.

Figure 12: Glucose on the paper first



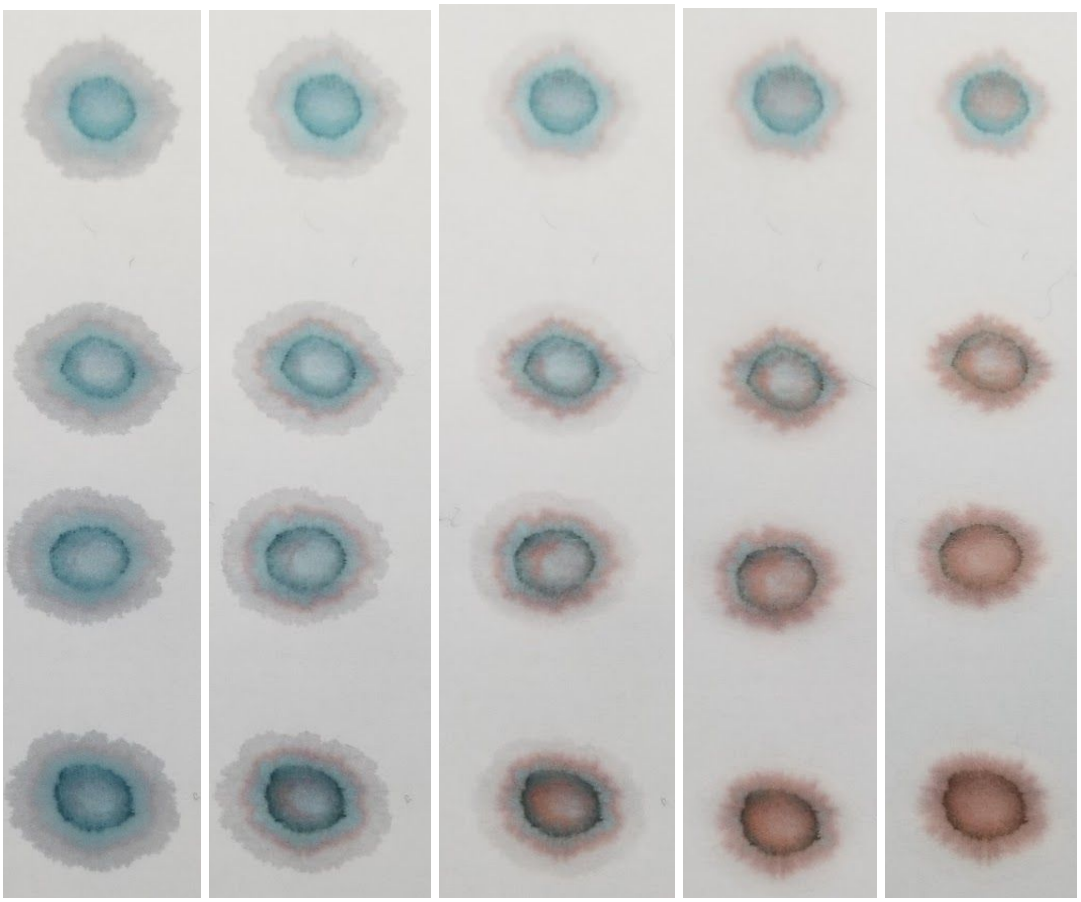
After choosing to start with glucose on the paper first, we tested with different amounts of glucose and the test solution. The tests with the bigger amounts lead to a larger amount of paper used because more of the solutions were soaked in. This was not ideal because the colors spread out too much. 5µL of both the glucose and the test solution was optimal as it conserved paper and spread to only a small area (Figure 13).

Figure 13: 5µL of Both Glucose and Test Solution: 70mg/dl, 130mg/dl, 150mg/dl, 230mg/dl concentration of glucose (left to right)



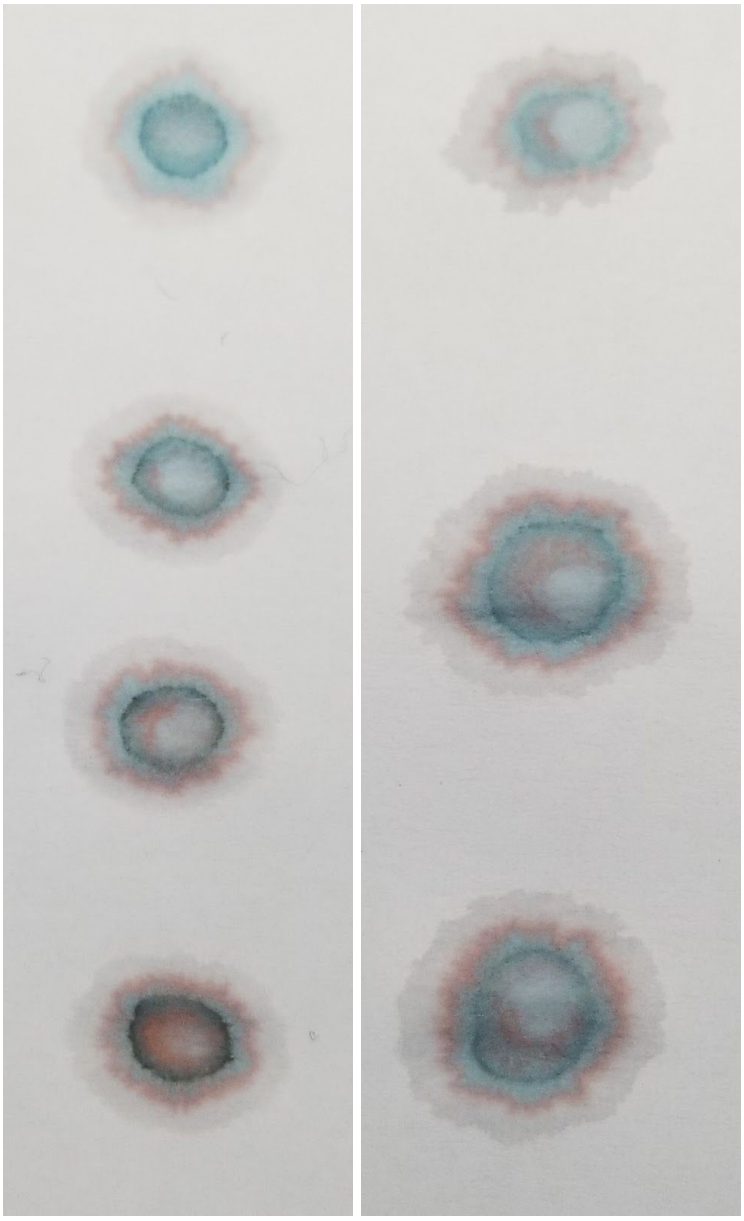
After finding the right amount to test, we had to test at what point should the diabetic take their reading. We took picture of the solutions at 1 minute intervals from 1-5 minutes (See Figure 14).

Figure 14: 5µL of Both Glucose and Test Solution at 1 minute intervals from 1-5 minutes (left to right): 70mg/dl, 130mg/dl, 150mg/dl, 230mg/dl concentration of glucose (Top to bottom)



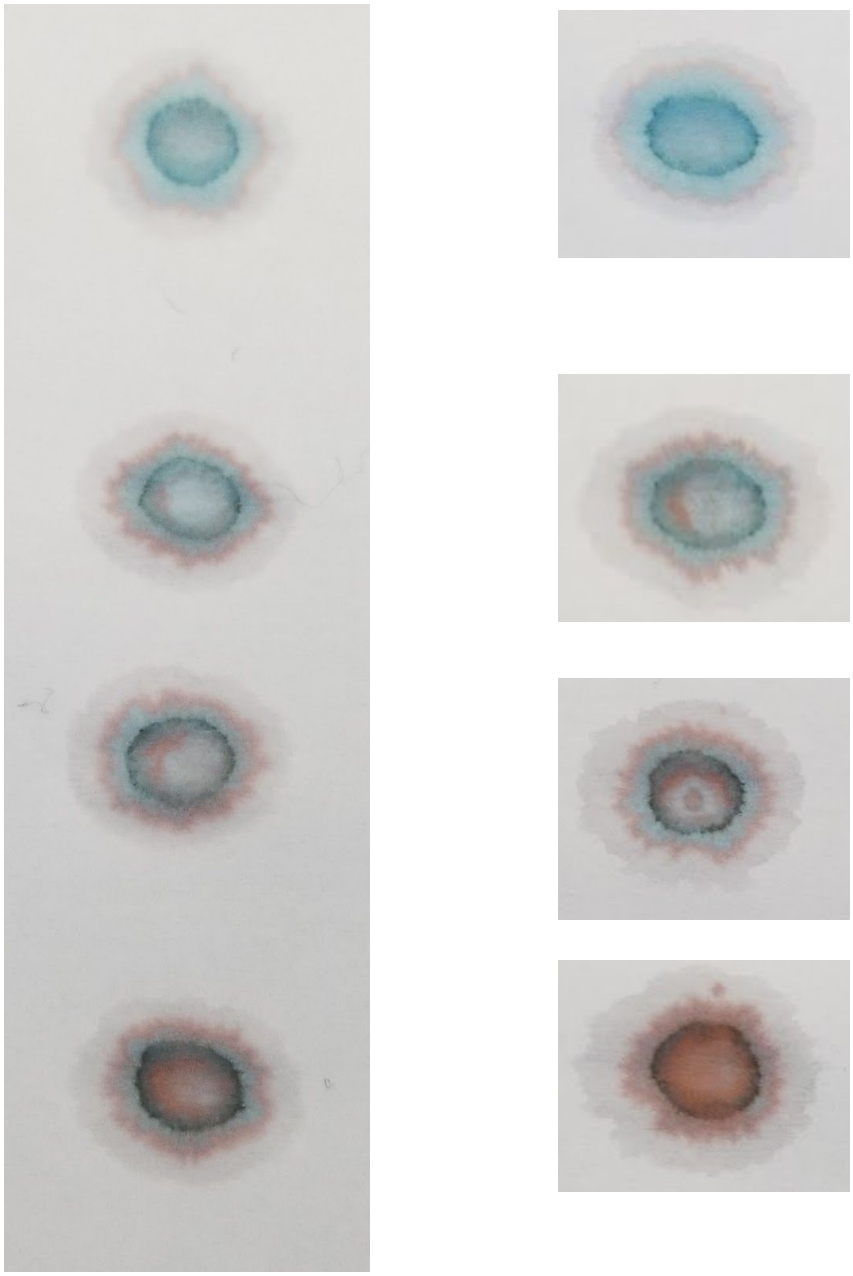
The 3 minute mark turned out have the best variation with both the blue and red color so it was chosen as the marking point of when to take the reading. We did further testing to make sure the tests was consistent we made Figure 13 the basis for our further tests. Glucose solutions with concentration 90mg/dl, 170mg/dl, and 190mg/dl were created to test how accurate of a reading you can get just by looking at the color (See Figure 15).

Figure 15: Comparison of basis glucose concentrations (left) with 90mg/dl, 170mg/dl, and 190mg/dl (top to bottom) glucose concentration (right)



It can be clearly seen that the 90mg/dl one goes between 70mg/dl and 130mg/dl while the 170mg/dl and 190mg/dl go between the 150mg/dl and 230mg/dl so the concentrations in between don't really have a problem but when you get close to the basis concentrations it can be hard to tell (see Figure 16).

Figure 16: Comparison of basis glucose concentrations (left) with another sample with the same concentrations (right) 70mg/dl, 130mg/dl, 150mg/dl, and 230mg/dl (top to bottom)



In the end the prototype was accurate to a certain degree because the closer you got to the basis concentrations the harder it was to tell what your concentration was but at these points the worst case scenario should be taken into account because if you are near the low or high end you are close to not being in the safe range so precautions should be taken no matter what. Further

testing would need to be done to make the prototype more accurate and also further testing would need to be done to see if the prototype could handle the temperature in Kampala Uganda as the test solution needs to be refrigerated and the temperature in the air could affect the test. Storage life of the test solution needs to also be tested because TMB is known to degrade over time.

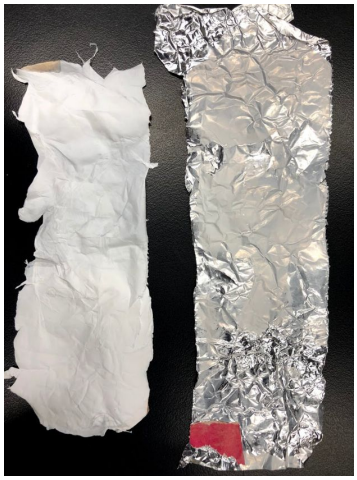
The TMB and Horseradish Peroxidase chemical reaction produces a color change ranging in the blue tones. Since blood contains a red pigmentation from hemoglobin, the addition of blood into the otherwise blue chemical reaction drastically changes the expected color as shown in Figure 17.

Figure 17. Solutions Mixed With Goat Blood

Therefore, a mesh designed to filter out the red blood cells was created from the polymer polycaprolactone (PCL). PCL was doused in acetic and formic acid for 24 hours to form a polymer solution. This solution was put into a syringe and placed into the handmade electrospinning machine in the IA Lab. Aluminum foil was placed onto the mantle of the electrospinning machine to catch the filament. The electrospun machine is designed to slowly push the syringe at a constant rate to slowly force out the polymer solution. The machine is connected to a 25,000 volt power supply which is used to convert the polymer solution into scattered filament. The random scatters of filament form together to form the red blood cell

mesh. The procedure to make the electrospun fibers were completed by Professor Weiser. An electrospun fiber mesh created in 60 minutes is shown in Figure 18.

Figure 18. Electrospun Fiber Mesh (60 minutes)



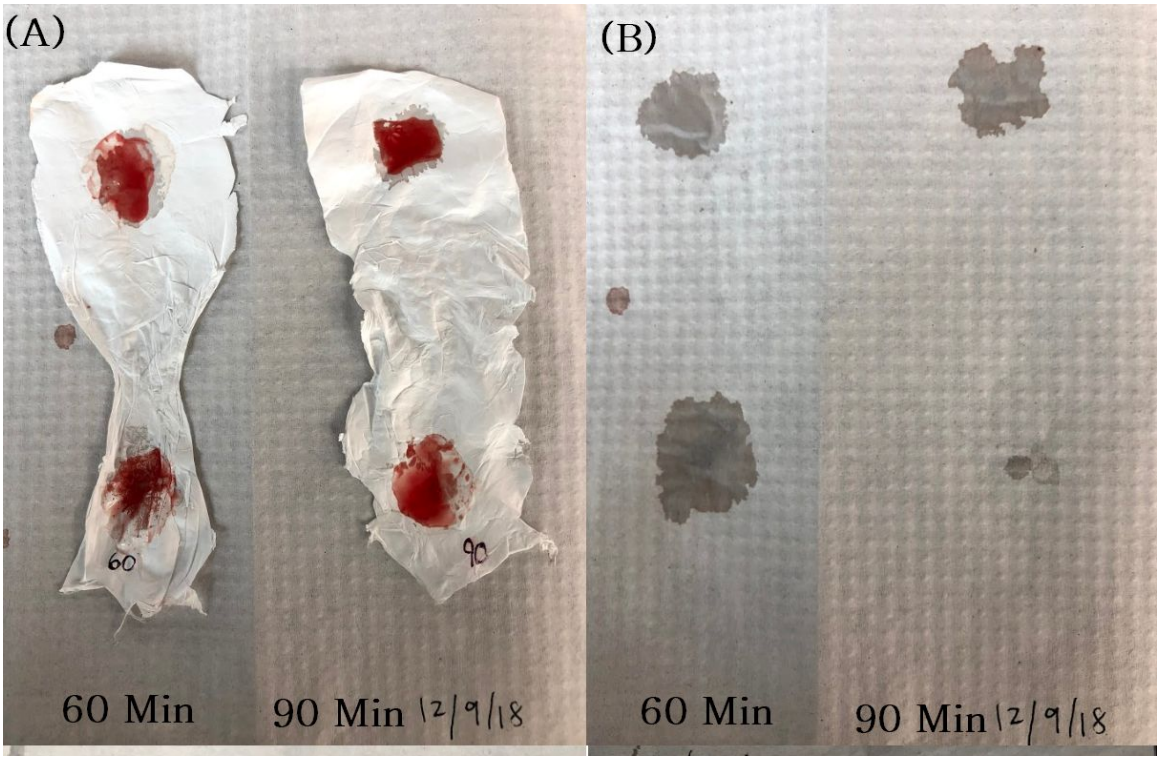
There were two factors taken into consideration when creating the electrospun fiber mesh: time and resistance. The longer the polymer solution was placed into the electrospinning machine, the thicker the resulting mesh and more resistant. However, the resulting mesh is also more narrow due to the additional layers of filament. Electrospun fibers have diameters in the nanometers whereas red blood cells have diameters of approximately 2 to 8 micrometers. It is unknown if the electrospun fiber mesh would filter out everything, not just the red blood cells, if additional layers of filament were added.

Considering time constraints, two electrospun fiber meshes created in 60 minute and 90 minutes were procured and used for initial testing. Due to issues with procuring blood, testing was significantly delayed. Initially, we wanted to use human blood but there were IRB concerns. Then, we wanted to use synthetic human blood but there were time constraints. As a result, goat blood was used to test.

A large amounts of goat blood was used to test if the blood could be filtered, as shown in Figure 19. The upper two squares in Figure 19 A and B show the filtration of goat blood by waiting for

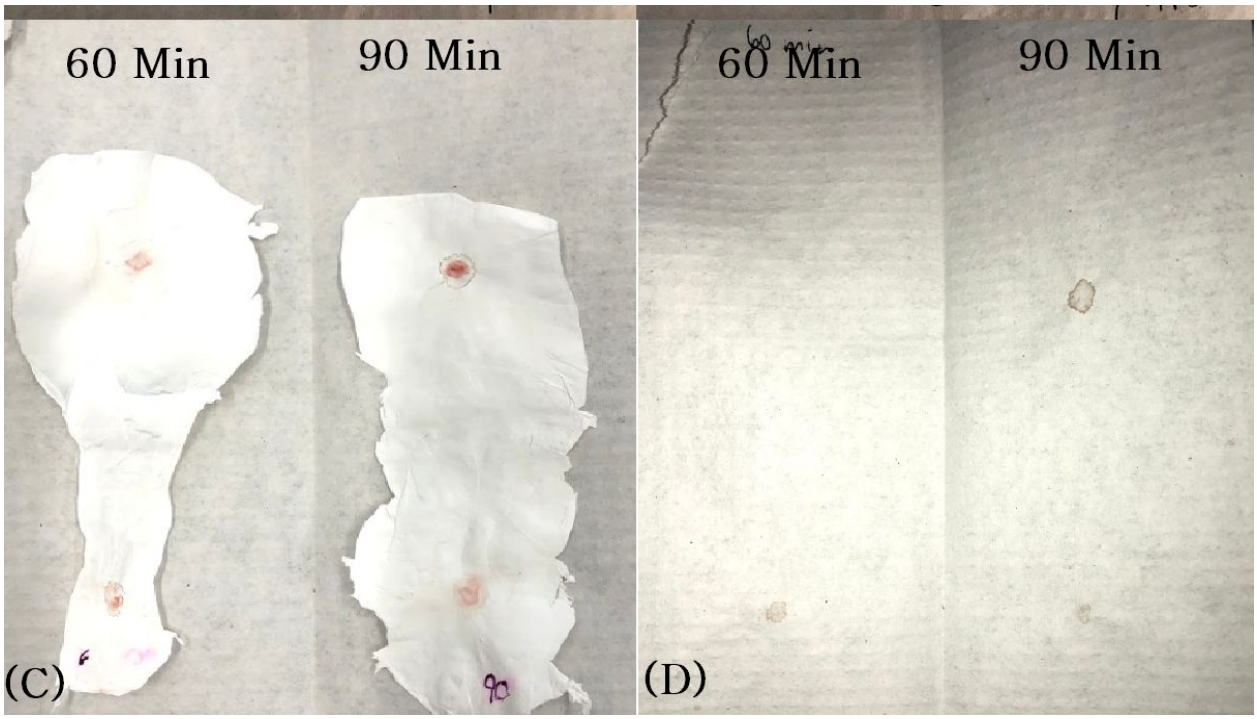
four minutes. The latter two squares show the filtration of goat blood by pushing a certain amount of blood using a finger. This is to mimic the actual procedure of diabetics pricing their finger with a lancet before placing it down on the filtration mesh.

Figure 19. Filtration of 200 µl of Goat Blood



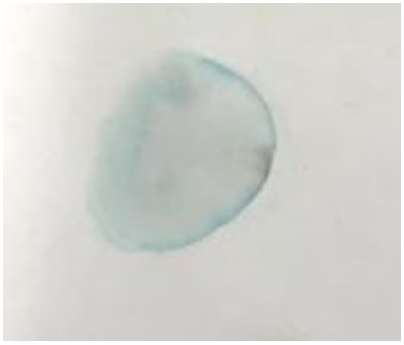
As shown above, the filtration was successful for 200 µl of goat blood. Within four minutes, the blood would naturally filter. Pushing the blood through for the mesh created in 60 minutes produced better results since more blood was filtered and the time spent to push was drastically shorter than the time spent on waiting. Although the pushing trial for the 90 minute mesh was worse than all the other results, more trials done and a more forceful push showed that the 90 minute mesh could attain the same results as a 60 minute mesh.

Figure 20. Filtration of 50 µl of Goat Blood



To mimic diabetic usage of the filtration mesh, 50 µl of goat blood was tested. As shown in Figure 20D, there were no splotches in the upper square of the 60 minute mesh which means even after four minutes of waiting, nothing was filtered out. In contrast, the 90 minute mesh was able to filter out some blood. Nevertheless, the trials performed when pushing the blood onto the filtration mesh of both the 60 minute and 90 minute mesh worked.

Figure 21. Filtered Goat Blood and Chemical Solution Result



The electrospun mesh was largely successful. It was tested in conjunction with the chemical solution as shown in Figure 21. The filtered goat's blood was filtered onto copy paper and a drop of the chemical solution was added in a 1:1:1 ratio of TMB: horseradish peroxidase: glucose oxidase. Multiple trials were performed and all of them resulted in the blue-ish color after five minutes.

BILL OF MATERIALS

The total allowed budget for the section was $450. Our team spent $201.01 on orders.

Table 6. Bill of Materials

Chemical	Model/CAS #	Part description	Supplier	Quantity	Cost
Tetramethylbenzidine (TMB)	MFCD00007748/ 54827-17-7	Redox indicator that can react with hydrogen peroxidase	Sigma-Aldrich	1g	$41.80
Horseradish Peroxidase (HRP)	MFCD00071339/ 9003-99-0	Enzyme needed in the TMB and hydrogen peroxidase reaction	Sigma-Aldrich	25kU	$122.59
Glucose Oxidase (GO)	MFCD00131182/ 9001-37-0	Reacts with glucose to create gluconic acid and hydrogen peroxide	Sigma-Aldrich	5kU	$22.55
Glucose ($C_6H_{12}O_6$)		Sugar	Amazon	500g	$8.07
Goat blood		Used to test the filter for filtering out of RBCs	L Aladdin Poultry	~1.5qt	~$10.00

Electrospun Fiber Mesh		Filters out RBC	Professor Weiser		~$0.01
				Total	~$205.02

ETHICAL CONSIDERATIONS

When coming up with a solution to any problem, it is important to remain moral, which is why one of our main agendas was to be ethical. Throughout the whole engineering design process, our team's actions and intentions followed the NYSPE Code of Ethics for Engineers ("Code of Ethics for Engineers", 2018).

Since our problem statement was focused in Kampala, we decided it was best to conduct research on that area to get an understanding of what is moral and accepted in their culture. Also, in order to get a true understanding of the scope of the project, we talked to people who went to Kampala to work with the medical centers. We were able to ask questions to people who first handedly experienced what it was like to live in Kampala.

After understanding the problem that we needed to solve, we began brainstorming and designing. During this process, we wanted a product that was safe for public use. As a result, we eliminated any chemicals that we did not find the material safety data sheet for or had little information to how safe it was, such as 5,6-Dimethylphenanthroline (Fe complex) ($C_{14}H_{12}N_2$) and 2,2`-Bipyridine (Fe complex) (C10H8N2). This limited our list of chemicals to TMB, horseradish peroxidase, and glucose oxidase so that is what we decided to move forthwith.

In addition, while testing, all data were recorded to make sure that none of our data were forged. It was important that we were honest in order to serve the best interest of the public. We did not omit any facts during the whole engineering process and none of the information was misrepresented. All of our data were recorded and plotted on a graph with a logarithmic regression. All the results of the blood glucose level on the app were based on many experiments.

In the future, for marketing the product, we plan to be honest and issue public statements in a truthful and objective manner. As the code of conduct for engineers goes, we would include all

relevant information in reports and be as truthful and straightforward as possible. This would ensure that we do not deceive the public and that they would trust the colorimetric test strips.

SAFETY

In any engineering project, safety is a major concern. Safety concerns on the preliminary designs, implemented chemical design, and testing procedures were noted throughout the entirety of this course and design of the colorimetric test strip.

The colorimetric test strip was chosen as the basis of our preliminary design. Many different indicators such as those containing metal oxides were eliminated due to potential toxicity to the environment and user. The chemical reaction selected consist of TMB and Horseradish peroxidase. Although both substances are classified as non-hazardous, ingestion, skin contact, and eye contact with these chemicals are to be thoroughly washed with water. To ensure safety, the design should be kept out of a child's reach. Therefore, as long as the chemicals are properly used for glucose regulation, the test strips are safe to use.

The test strips are an invasive strategy to measuring blood glucose level. Blood must be used on the test strip for proper glucose measurement. This usage of blood is a large potential hazard but can be alleviated with the proper protocol. Firstly, the amount of blood necessary for the functioning of the colorimetric test strip can be lessened by increasing the amount of chemical indicator on the test strip. Secondly, the blood is filtered through the filtration mesh and the mesh is removed before any chemical addition to prevent cross-contamination. Although red blood cells can be washed from the filtration mesh with tap water, chemicals cannot. Later placement of an open wound with a mesh with with potential chemical residues is a serious safety hazard. To prevent this, proper instructions on how to use the strips will be outlined and preferably, demonstrated to the diabetics in Kampala. It would be better if patient education is spread throughout Kampala to raise awareness, provide practice to lessen mistakes of using the test strips, and thereby lessen risks of biocontamination.

The design prototype was created and tested in laboratory settings with supervisors in proximity. A draft of the laboratory procedure and all relevant SDS sheets were procured and sent to

Professor Savizky for safety approval. Chemical testing done in the Kanabar Lab were under the supervision of Professor Jajuvensic and proper chemical rules were followed. Chemicals that needed refrigeration such as glucose oxidase were properly refrigerated after usage. The production of the red blood cell filtration mesh through electrospinning was done in the IA Lab, under the supervision of Professor Weiser. Animal blood was used as a substitute for human blood during the red blood cell filtration tests. All chemicals and materials were properly labelled, sealed, and stored to avoid contamination and safety hazards.

PROJECT ONLINE DELIVERABLES

Link to website and video: https://theregulators.github.io/
Link to final presentation: https://goo.gl/PH2ayu

The website was created as soon as the solution was narrowed down to the colorimetric test strip, and relevant content was added throughout the development of the product. The most significant improvements and additions to the site were made during the week of midterm and final presentations. The website was developed using the React Javascript framework, and its design was intended to be navigationally intuitive and minimalistic. All content on the website, including documents and media, are original content generated from this project.

After the solution was created and refined, we began the process of making a video to inform consumers and suppliers of the product we made. The video was made at the end of the term using clips and pictures taken throughout the semester. The video was stitched together using iMovie.

CONCLUSIONS AND FUTURE CONSIDERATIONS

The finished product, while still a rough prototype, showed a color change roughly corresponding to change in blood glucose levels. While not as precise as the traditional glucometer and test strips, it is a viable means of rough estimation of blood glucose levels and would be of good use for those living in developing nations, such as Uganda. The color change that the glucose oxidase-TMB-HRP solution produced would be separated into three categories: low, medium, and high, which was represented by a light blue, medium blue, and dark blue-red color respectively. This would be enough to let a diabetic know approximately what their blood glucose level was. While the app was not as effective in precisely reading the exact blood glucose level from the color change produced by the test strip, it was still fully constructed and with some modifications, further testing, and more time, would definitely be able to produce more valid results.

The entire process was done ethically; no results were forged. Diabetes is a serious issue and any mismanagement could result in serious injury or possibly death. Therefore, calculations were done precisely and with the utmost care. The project was also done with the goal of helping people in developing nations, so it was made to be as cost-effective as possible, to a reasonable degree of accuracy. The cost analysis showed that the price of a strip averaged out to be less than a cent per unit. This was considered to be a big success and would surely help those in Kampala. Additionally, the product would be packaged safely in an airtight bottle which would prevent the decay of the chemicals inside. With diabetes already being such a serious disease, it was important that the chemicals in the strip would not be harmful to the patient.

While the project appeared to be overall successful— safely and inexpensively measuring blood glucose level in a consistent manner— there were many areas open for future research and improvements to the design. Some of the major future considerations are listed below.

For portability reasons, it would be a good idea to work on the combination of all of the parts. The current design consists of many separate components, which would be difficult to distribute and confusing to the end user. Distributing the solution combined with the strip may be a better solution. A way to extend the shelf life of the solution is also an important consideration—storage of the chemical solution in refrigerated amber-colored glass bottles provides a shelf life of roughly one year. This is additional work for the user and therefore not ideal.

In terms of the mobile application, while a large improvement over the 2017 group's analysis algorithm, there are several improvements that can be made to improve the color analysis. More research into the colors and patterns corresponding to different blood glucose levels is necessary to determine an accurate parameters for color analysis. Future research into machine learning to find these parameters, as well as ways to take into account lighting and placement of the item, would be very beneficial to the algorithm. The current estimate of ±30mg/dL variation for the app algorithm only applies to a single lighting condition (and worse with changes of lighting or shadows), and the running time of the algorithm takes 10 to 20 seconds on an average Android tablet; it is hopeful that machine learning can be used to improve both of these statistics.

Currently, the mobile application is somewhat barebones and does not implement any storage or data analysis of past BGL measurements. It is possible to create a tab of the app that displays past glucose measurements and sets a goal for daily sugar intake so that a patient could track their daily intake and know how much more sugar they could consume for the day, much like apps such as My Fitness Pal. The about page should be updated with more captivating and intuitive how-to visuals, especially for Kampalans who cannot read English; better yet, there should be multiple translations of the text, to the other languages most often spoken in Kampala, that can be switched between in the app.

The test chemical's concentrations were not properly tested to find the most optimal and cost effective levels. The current concentrations were based on values for R&D assays and such are most likely higher than necessary. In the future, the concentrations can be tested to find the

lowest amount of each reagent possible while still keeping the reliability and actual function of the chemical mixture.

ACKNOWLEDGEMENTS

We would like to thank Professor Raja, Dean Savizky, Professor Weiser, and Professor Janjusevic, Revans Ragbir, Beth Slack, Members of the Albert Einstein Medical Center, Members from Johnson & Johnson, and Paulo Serodio from the 2017 EID-101 Section E group for their expert opinions and feedback.

We would like to give special recognition to Professor Jajuvesic and Professor Medevedik for allowing us to use the Maurice Kanbar Lab during their free time and advising our project. We would also like to give special recognition to Professor Weiser for specially manufacturing electrospun polymers at the expense of her time.

We would also like to thank Carolina Biological Supply Company for answering our questions about blood and for Sigma Aldrich for providing materials used.

Additionally, we would like to thank the 2017 Section-E EID group, G Cubed Solutions, for allowing us to build off of the idea of colorimetric strips.

REFERENCES

Bergmeyer, Hans Ulrich, et al. *Methods of Enzymatic Analysis*. 2nd ed., vol. 1, Academic Press, Inc., 1974.

"Blood Sugar Levels Chart." *Diabetes Alert*, 25 June 2015, diabetesalert.net/blood-sugar-levels-chart/.

Chan, Michael, et al. *Colorimetric Test Strip and Mobile Application For the Measurement of Blood Glucose*. 2017, The Cooper Union for the Advancement of Science and Art.

Code of Ethics for Engineers. National Society of Professional Engineers, July 2018.

Ercolanelli, Léo. "How We Tackled Color Identification." *Algolia*, Algolia, 25 Jul. 2016, https://blog.algolia.com/how-we-handled-color-identification/.

Jensen, Sarah. "How do glucometers work?" *MIT School of Engineering*, MIT, 18 Oct. 2011, https://engineering.mit.edu/engage/ask-an-engineer/how-do-glucometers-work/.

Uganda Bureau of Statistics. *Uganda National Household Survey 2012-2013*. Kampala, Uganda: Uganda Bureau of Statistics.

Uganda Bureau of Statistics. (2017, December 19). *The Uganda National Household Survey 2016/17*. Retrieved October 1, 2018, from https://www.ubos.org/onlinefiles/uploads/ubos/pdf documents/2017_UNHS_26092017-Final_Presentation.pdf

University of Bath. "Bloodless revolution in diabetes monitoring: Scientists have created a non-invasive, adhesive patch, which promises the measurement of glucose levels through the skin without a finger-prick blood test." *ScienceDaily*, ScienceDaily, 9 April 2018, www.sciencedaily.com/releases/2018/04/180409170952.htm.

Vermeiren, Karolien & Van Rompaey, Anton & Loopmans, Maarten & Eria, Serwajja & Paul, Mukwaya. (2012). *Urban growth of Kampala, Uganda: Pattern analysis and scenario development. Landscape and Urban Planning*. 106. 199-206. 10.1016/j.landurbplan.2012.03.006.

The National Population and Housing Census 2014 – Area Specific Profile Series, Kampala, Uganda. Kampala Capital City Authority, 2017.

APPENDIX I: MOBILE APP CODE

The overall concept for the mobile application is outlined in the Design Documentation, Section III. The mobile application was written in Java for Android, using the Android Studio IDE, but the concepts can be extended to other platforms. Only snippets of code relevant to the image processing will be provided below. Minor visual changes and comments may have been modified for presentation purposes. The full app code is open source and available on GitHub at https://github.com/theregulators/regulators-android.

I. UI Thread Controller

The code shown in Snippet 1 is that running on the main (UI) thread. This thread calls the drawing function, and already has the time-intensive task of drawing the camera input to a `TextureView` every frame. The image analysis takes a long time, so to prevent lockups, a secondary thread is created when the image processing is taking place. A flag (`parseBitmapLock`) is set whenever image processing begins and unset when it finishes, in order to prevent multiple image processing events to overlap, causing larger processor strain.

A bitmap of the camera input is sent to `ColorDetection.getColor()` (stage one), which returns the average color of the chosen cluster (discussed in the Design Documentation). This color is then sent to `BGLDetermination.colorToBGL()` (stage two). When the processing is complete, a request is sent back to the UI thread to update the BGL reading.

Snippet 1. UI Thread Controller (ScanFragment.java)

```
public volatile static boolean parseBitmapLock = false;
public volatile static String bglTextViewText = "---";
private void parseBitmap() {
```

```
  // lock while running to avoid processor strain
  if(ScanFragment.parseBitmapLock) {
    Toast toast = Toast.makeText(getContext(),
        "An analysis is already in progress!",
        Toast.LENGTH_SHORT);
    toast.show();
    return;
  }
  bglTextView.setText("Analyzing...");

  new Thread(new Runnable() {
    @Override
    public void run() {
      // set lock flag
      ScanFragment.parseBitmapLock = true;
      // get bitmap
      Bitmap bitmap = textureView.getBitmap();
      if(bitmap == null) return;
      int width = bitmap.getWidth();
      int height = bitmap.getHeight();
      int[] pixels = new int[width * height];
      bitmap.getPixels(pixels, 0, width, 0, 0, width, height);
      for(int i = 0; i < 100; i++) {
        int j = pixels[i];
      }
      // perform stage 1
      VectorRGB averageColor =
        ColorDetection.getColor(pixels, width, height);
      // perform stage 2
      double bgl = BGLDetermination.colorToBGL(averageColor);
      // write output to UI
      bglTextViewText = "" + (Math.round(bgl * 10.0) / 10.0);
      getView().post(new Runnable() {
        @Override
        public void run() {
          bglTextView.setText(bglTextViewText);
        }
      });
      // remove lock
      ScanFragment.parseBitmapLock = false;
    }
  }).start();
}
```

The snippet above uses a helper class for colors, `VectorRGB`. This class was implemented as shown in Snippet 2. (It includes some functions from the first iteration of the image analysis that are not used in the current design. The original design included more work with vectors and projections, which may also be used in future iterations of the current design.)

Snippet 2. `VectorRGB` Helper Class (VectorRGB.java)

```
public class VectorRGB {
  public double r;
  public double g;
  public double b;
  public VectorRGB(double r, double g, double b) {
    this.r = r;
    this.g = g;
    this.b = b;
  }
  public VectorRGB(int packedInt) {
    this.setColor(packedInt);
  }
  public VectorRGB(int[] componentArray) {
    this.r = componentArray[0];
    this.g = componentArray[1];
    this.b = componentArray[2];
  }
  public void setColor(int packedInt) {
    this.r = Color.red(packedInt);
    this.g = Color.green(packedInt);
    this.b = Color.blue(packedInt);
  }
  public double dot(VectorRGB v2) {
    return r*v2.r + g*v2.g + b*v2.b;
  }
  public double norm() {
    return Math.sqrt(r*r + g*g + b*b);
  }
  public VectorRGB negate() {
    return new VectorRGB(-r, -g, -b);
  }
  public VectorRGB add(VectorRGB v2) {
    return new VectorRGB(r+v2.r, g+v2.g, b+v2.b);
  }
  public VectorRGB subtract(VectorRGB v2) {
```

```
    return this.add(v2.negate());
  }
  public double distanceTo(VectorRGB v2) {
    return this.subtract(v2).norm();
  }
  public VectorRGB timesScalar(double k) {
    return new VectorRGB(r*k, g*k, b*k);
  }
  public VectorRGB projOn(VectorRGB v2) {
    return v2.timesScalar(this.dot(v2) / Math.pow(v2.norm(), 2));
  }
  public VectorRGB ortProjOn(VectorRGB v2) {
    return this.subtract(this.projOn(v2));
  }
  public String toString() {
    return String.format("Vector RGB R: %f G: %f B: %f", r, g, b);
  }
  public int toColorInt() {
       int a = 255;
    return (a & 0xff) << 24 | ((int) r & 0xff) << 16 | ((int) g &
0xff) << 8 | ((int) b & 0xff);
  }
}
```

II. Color Detection (Stage One)

The description of the color detection code in Snippet 3 is outlined in the Design Documentation.

Snippet 3. Color Detection (ColorDetection.java)

```
public class ColorDetection {
  private static class Pixel {
    public int x;
    public int y;
    public Pixel(int x, int y) {
      setPos(x, y);
    }
    public void setPos(int x, int y) {
      this.x = x;
```

```
      this.y = y;
    }
  }

  private static class PixelCluster {
    public int pixelCount;
    public VectorRGB color;
    public List<Pixel> pixelList = new ArrayList<>();
    public PixelCluster(int r, int g, int b) {
      color = new VectorRGB(r, g, b);
    }
    public void addPixel(Pixel pixel) {
      pixelList.add(pixel);
      pixelCount++;
    }
  }

  // main color analysis function
  public static VectorRGB getColor(int[] bitmap, int width, int
height) {
    // step 1: initializing pixel clusters
    PixelCluster[][][] pixelClusters = new PixelCluster[16][16][16];
    int i, j, k, x, y;
    for(i = 0; i < 16; i++) {
      for(j = 0; j < 16; j++) {
        for(k = 0; k < 16; k++) {
          pixelClusters[i][j][k] = new PixelCluster(i << 4, j << 4, k
<< 4);
        }
      }
    }
    // step 2: filling pixel clusters
    VectorRGB color = new VectorRGB(0);
    for(y = 0; y < height; y++) {
      for(x = 0; x < width; x++) {
        color.setColor(bitmap[y * width + x]);
        int thresholdR = (int) color.r >> 4;
        int thresholdG = (int) color.g >> 4;
        int thresholdB = (int) color.b >> 4;
        Pixel pixel = new Pixel(x,y);
        pixelClusters[thresholdR][thresholdG][thresholdB]
          .addPixel(pixel);
      }
    }
```

```
    // step 3: analyzing pixel clusters
    int lowThresholdCount = width * height / 1000;
    int highThresholdCount = width * height / 2;
    int thresholdNum = 0;
    List<PixelCluster> chosenClusters = new ArrayList<>();
    for(i = 0; i < 16; i++) {
      for(j = 0; j < 16; j++) {
        for(k = 0; k < 16; k++) {
          // get pixel cluster
          PixelCluster currentPixelCluster = pixelClusters[i][j][k];
          // filter out clusters with too few pixels
          if(currentPixelCluster.pixelCount < lowThresholdCount ||
currentPixelCluster.pixelCount > highThresholdCount) {
            // filter condition 1
            continue;
          }
          // check "jump" method
          int xAvg = 0;
          int yAvg = 0;
          for(Pixel clusterPixel : currentPixelCluster.pixelList) {
            xAvg += clusterPixel.x;
            yAvg += clusterPixel.y;
          }
          xAvg /= currentPixelCluster.pixelCount;
          yAvg /= currentPixelCluster.pixelCount;
          if(Math.abs(xAvg - width/2) > width/4 ||
             Math.abs(yAvg - height/2) > height/4) {
            // filter condition 2
            continue;
          }
          double error = 0;
          for(Pixel clusterPixel : currentPixelCluster.pixelList) {
            error += Math.sqrt(Math.pow(clusterPixel.x - xAvg, 2) +
              Math.pow(clusterPixel.y - yAvg, 2));
          }
          double relativeError = error /
            currentPixelCluster.pixelCount;
          if(relativeError > 150) {
            // filter condition 3
            continue;
          }
          if(k >= i && k >= j && chosenClusters.size() < 4) {
            chosenClusters.add(currentPixelCluster);
          }
```

```
        }
      }
    }
    VectorRGB avgBlue = new VectorRGB(0, 0, 0);
    int totalCount = 0;
    for(PixelCluster pixelCluster : chosenClusters) {
      totalCount += pixelCluster.pixelCount;
      avgBlue = avgBlue.add(new VectorRGB(pixelCluster.color.r,
        pixelCluster.color.g,
        pixelCluster.color.b).timesScalar(pixelCluster.pixelCount));
    }
    if(totalCount != 0) {
      avgBlue = avgBlue.timesScalar(1.0 / totalCount);
    }
    return avgBlue;
  }
}
```

III. Color Analysis (Stage Two)

The blood glucose level determination is also described in the Design Documentation. The functions `rToBGL()`, `gToBGL()`, and `bToBGL()` are the inverse functions of the three calibration trendlines in Figure 2.

Figure 4. Blood Glucose Level Determination from Color (BGLDetermination.java)

```
public class BGLDetermination {
  final static private VectorRGB colorStart =
    new VectorRGB(255, 0, 255);
  final static private VectorRGB colorEnd =
    new VectorRGB(255, 255, 0);

  // these coefficients of determinations and functions
  // are experimentally determined
  // see the calibration curves for more information
  private static double rToBGL(double r) {
    return Math.exp((112.041-r)/13.921) * 10;
  }
```

```
  private static double gToBGL(double g) {
    return Math.exp((159.955-g)/28.059) * 10;
  }
  private static double bToBGL(double b) {
    return Math.exp((165.499-b)/28.447) * 10;
  }
  private static double rR2 = 0.624163;
  private static double gR2 = 0.853277;
  private static double bR2 = 0.887773;

  public static double colorToBGL(VectorRGB color) {
    double rBGLGuess = rToBGL(color.r);
    double gBGLGuess = gToBGL(color.g);
    double bBGLGuess = bToBGL(color.b);
    double weightedBGLGuess = (rBGLGuess * rR2 + gBGLGuess * gR2 +
bBGLGuess * bR2) / (rR2 + gR2 + bR2);
    return weightedBGLGuess;
  }
}
```