A Point-of-Care Device for Measuring Glucose, ketones, hemoglobin, and Glycated Hemoglobin From Whole Blood

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A Point-of-Care Device for Measuring Glucose, Ketones, Hemoglobin, and Glycated Hemoglobin from Whole Blood

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A Thesis in the Field of Bioengineering and Nanotechnology
for the Degree of Master of Liberal Arts in Extension Studies

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Abstract

Point-of-Care (POC) blood glucose meters and test strips are utilized by millions of individuals multiple times daily. Unlike clinical laboratories, which analyze plasma samples for a true glucose value, POC devices use whole blood. Whole blood values can differ from plasma values by as much as +/− 20% of glucose concentration values. Aside from utilizing whole blood for glucose measurements, the biosensor test strips use embedded electrodes, and enzymes such as glucose oxidase and glucose dehydrogenase. The glucose measurements using these test strips are further affected by free oxygen, and the presence of other electroactive species in the blood sample. Biofouling of the test strips by materials such as proteins and chloride ions in blood further compromise the accuracy and precision of glucose measurements. This study presents an alternative detection system to the test strips and glucose meters currently on the market. We incorporated a three-layer paper microfluidic device to separate blood cells and proteins from smaller molecules such as glucose and ketones in layered system using filtration. We developed assays to measure the concentrations of glucose and ketones using fluorophores spotted in specific testing areas for glucose, ketones and red blood cells. We envision, once fully developed, these paper microfluidic-based assays will accurately and precisely quantitate glucose and ketone concentrations. Using the red cell count, calculations of the hemoglobin, hematocrit, and glycated hemoglobin (A1C) will be made. The glucose meter will possess a CCD camera for quantitation of
each sample, and compare the sample concentrations to that from programmed standard curves. In our laboratory studies, we were able to perform the standard curves for glucose, glucose oxidase and ketones. The results of our work was inconclusive due to the lack of a material holder for the spectrophotometer we were utilizing, which would have measured the fluorescence of the individual testing areas that had been pre-spotted with the corresponding fluorophores. However, proof of concept for separation of blood cells from plasma was demonstrated. Future work will include repeating the previous methods using a material sample holder on a spectrophotometer, and the fabrication of the paper microfluidic devices using a laser cutter, a wax printer, and a laminator.
Dedication

For Karl and Skylar
Acknowledgments

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Chapter I

Introduction

In the year 2014 there were 387 million people living with diabetes, type one or type 2 (International Diabetes Federation Diabetes Atlas, 2014). The number of cases of diabetes is expected to increase by 205 million by the year 2035 (International Diabetes Federation Diabetes Atlas, 2014). Diabetes is a global killer, rivaling HIV/AIDS in its mortality – more than 4 million people a year die from this illness – and millions suffer from complications such as damage to the heart, blood vessels, kidneys, eyes, nerves, and teeth. Diabetics also have higher risks of developing severe infections and foot ulcers that often lead to lower leg amputations. This illness is the leading cause of blindness among adults in the United States between the ages of 20-74 (approximately 12,000 to 24,000 cases per year) and end-stage renal disease, which accounts for 35% of dialysis and kidney transplants (Heller & Feldman, 2008).

Frequent and rapid blood glucose monitoring is imperative. The only way patients can monitor their blood glucose is through home testing using a self monitoring blood glucose meter (SMBG), and adjusting their medication along with medical follow-up. Although SMBGs have greatly helped patients monitor their condition, they are not perfect. In-home glucose readings differ from central laboratory (CL) values by as much as 20% (personal communication), as these devices use whole blood not blood plasma as in a CL. A precise and accurate meter’s
values closely reflect the reference value, a measure that compares meter results against CL methods that use plasma-based samples (Tonyushkina & Nichols, 2009). The American Diabetes Association (ADA) recommends that the accuracy of a glucose point-of-care (POC) assay be less than 5% of a CL value, but this has yet to be achieved (Yoo & Lee, 2010). The goal is to develop a glucose meter with less than a 5% error rate compared to that of a CL value, and also include tests for hematocrit, ketones, and glycated hemoglobin (A1C). Clinical pathologists Boyd and Bruns (Boyd & Bruns, 2001) have suggested that SMBGs require accuracy of 2% or less to avoid excessive hypo- and hyperglycemia events. To date, SMBGs are not standardized and calibrated according to the ADA’s suggested guidelines.

Currently the predominant type of biosensor test strip for glucose detection uses embedded electrodes, resulting in a sensitive, easily reproducible and low-manufacture cost product (Newman & Turner, 2005). The most commonly used enzymes for glucose detection are glucose oxidase (GOx) and glucose dehydrogenase (GDH). Glucose measurements in test strips are hampered by the use of free oxygen as a catalytic mediator and the presence of other electroactive species in the blood. Biofouling from materials on test strips such as blood proteins and chloride ions occur on meter electrodes, reducing their efficacy (Toghill & Compton, 2010).

A relatively new and alternative approach to aid in the control of diabetes is the use of continuous glucose monitors (CGM). Using an ultra-thin needle inserted under the skin it measures glucose levels throughout the day. An alarm will sound when glucose levels are out of range, too high or too low (Joslin Diabetes Center
Newsletter, 2016). However, there are two major drawbacks to this approach. First, the sensor can cause discomfort to the patient and needs to be replaced every three to seven days. Also, the sensor must be synced with the patient’s glucose meter on a frequent basis. Moreover, the CGM has to be calibrated and continuously monitored via finger stick and glucose meter checks. CGM only alerts the patient to very high or very low readings, but it is still the glucose meter that will give the glucose concentration to the patient for self-treatment.

Implantable insulin pumps (also known as an “artificial pancreas”) are now in the development stage. However, without accurate and precise glucose meters the medical consequences of inaccuracies of SMBGs will become even more apparent. Nevertheless, insulin pumps will still require SMBGs to calibrate, and continuously monitor their function.

Unfortunately after decades on the market and in daily use, glucose test strips and meter technology has not evolved to the point that a patient and/or physician can feel confident in their readings. Glucose readings from a CL seem to be the only accurate and precise measurements one can count on, but it is unrealistic to think that multiple daily glucose concentrations can be performed at these laboratories.

Glucose meters will not disappear from patient monitoring even with CGM or an artificial pancreas, as they are needed for calibration and monitoring of function. Until there is a true cure for diabetes, the glucose meters will continue to be used. Currently the call by the ADA for standardization of the outputs and for the lowering of the error rates to 5% or less finally set the bar for all the manufacturers of glucose
meters to achieve these goals, which should have happened decades ago (Boyd and Bruns, 2001).

There are several factors and sources of potential errors that need to be overcome in order to achieve the goals mentioned above. They are:

- Accuracy and precision
- Variability in test strips and glucose meters
- Conversion of whole blood glucose values to plasma values
- Physical factors

Accuracy and Precision

Precision and accuracy must both be present so that glucose values accurately reflect that of a reference value based on CL. The technical accuracy of a glucose meter, which also includes the test strip, is defined by comparing a meter’s result with that of a CL reference that uses plasma/serum based samples (Tonyushkina & Nichols, 2009). The enzyme hexokinase is used in many CL as a reference method for measuring glucose via spectrophotometry (Yoo & Lee, 2010). Prior to 2009 an American Diabetes Association (ADA) statement recommended that the acceptable error for point-of-care glucose monitoring devices (POCGMDs) from all sources (user, analytical, etc.) should be less than 10% for glucose ranging from 30-400mg/dl at all times (Rebel et al., 2012, Ginsberg, 2009). It went further to recommend that glucose measurements should not differ more than 15% from values obtained by a laboratory reference method. In 1996 the ADA decreased the maximum allowable analytical error to <5%. Giordano and colleagues (1989)
compared several POCGMDs with a reference method and found only three devices had acceptable measurement accuracy. The remaining four devices consistently underestimated blood glucose levels of less than 100mg/dl. Chen and colleagues (2003) blindly evaluated four POCGMDs, and found none achieved the ADA 1996 recommendations for accuracy. Seven POCGMDs, which involved four different manufacturers, were compared to a reference method by Khan et al. (2006) and only one device met the ADA 1996 performance requirements. The major concern was the disagreement with reference values within the critical hypoglycemic and hyperglycemic ranges, which could result in an adverse clinical decision. At the extreme glucose values in the hyperglycemia and hypoglycemia ranges, when compared to CL devices, 61% of values differed more than 10% from a reference method. An alarming 57% of measurements differed by more than 20% in the hypoglycemic range. Freckmann et al. (2010) tested twenty-four POCGMDs and found more than 40% of the devices did not meet the minimum accuracy requirements of DIN EN ISO 15197:2003-Deutsches Institut fur Normung (German Institute for Standardization). International Standards Organization (ISO) 15197-2003 specifies requirements for in vitro glucose monitoring systems that measure glucose concentrations in blood samples via self-testing devices. This is applicable to manufacturers of such devices and regulatory authorities for the responsibility of assessing performance of these devices) these results are alarming, and make managing such a serious illness very difficult. SMBGs results do not reflect the inaccuracies inherent in them to a patient thereby making their treatment decisions possibly life threatening. The device we propose to develop will use paper
microfluidics employing a separation methodology by use of sequential filtration. The cellular components will be separated from the proteins and smaller molecules separated from the proteins. Using the cellular components the red blood cells can be selectively labeled with a fluorophore and quantitated for the hemoglobin concentration, which can further calculate hematocrit for a true glucose value, and the A1C value. Using fluorescence which is extremely quantitative, standard curves for glucose will be generated and programmed into the software of the device, as well as a standard curve for a negative control, mannose.

Variability in Test Strips and Glucose Meters

Test strips are manufactured in large batches, which allows for strip-to-strip variation. Variation in the coverage of the enzymes on the test strip adds error and can lead to underestimation or overestimation of glucose values. Likewise, a reduction of the mediator will also cause errors, as there is not enough to react with the enzyme. Inconsistencies in machining test strips translate into inaccuracies in blood glucose readings. The size of a test strip is approximately 1mm wide, 3mm in length, 0.1mm thick, and sample volume channel holds ~0.1ul of blood (Heller & Feldman, 2008). The sample strips are appreciably small so any variation of volume in the sample channel will cause a large difference in sample quantitation. A sample well-size variation of just 50µm will add an additional 3% error (Ginsberg, 2009).

There are two major enzymes used in SMBGs: glucose oxidase (GOx), and glucose dehydrogenase (GDH). The testing strips are electrochemical biosensors that have enzymes, co-factors, and sometimes mediators on them that interact with
glucose in a blood sample. There are usually two electrodes on test strips: 1) a working electrode where current is passed through it and an electrochemical (redox) reaction takes place, and 2) a reference electrode where no current is passed or no reaction occurs. An electrode inside the meter measures the current produced by the redox reaction after the test strip is inserted into the device. The meter measures the output voltage from the test strip and calculates the glucose concentration. The glucose value is then converted into a digital output (Vahist, 2011). GOx requires a cofactor, flavin adenine dinucleotide (FAD) that acts as an electron acceptor and is then reduced to FADH2 (Yoo & Lee, 2010).

\[
\text{glucose} + \text{GOx-FAD}^+ \rightarrow \text{glucolactone} + \text{GOx-FADH}^2
\]

\[
\text{GOx-FADH}^2 + \text{O}_2 \rightarrow \text{GOx-FAD}^+ + \text{H}_2\text{O}_2
\]

The oxidation of glucose occurs with the reduction of FADH2 to the oxidized form, FAD+. (Hilditch & Green, 1991). GOx competes with oxygen from red blood cells, as the hematocrit increases, as will the oxygen concentrations. This is particularly important for patients receiving oxygen therapy for other disease processes. The effect of increased oxygen is important because the mechanism by which this biosensor works is that increased oxygen levels will replace the oxygen of the mediator in the reaction. Oxygen is an electron acceptor and will compete with the electron-transporting mediator for reduced glucose oxidase/FADH on the test strip (Tang et al., 2001). Using oxidases where peroxide is produced will affect glucose measurements due to fluctuations in oxygen concentrations. In a blood sample so small, usually with a volume of 1µl or less, there is not enough oxygen to maintain glucose oxidation. This in turn leads to inaccuracies in glucose detection.
Endogenous or exogenous substances in the blood can also cause errors as well. Triglycerides can interfere with GOx and cause inaccuracies by taking up volume, decreasing the amount of glucose; hence glucose values will be reported lower (Heller & Feldman, 2008, Ginsberg, 2009). The electrode can oxidize uric acid and give falsely elevated glucose levels. Other factors that affect the GOx reaction are drugs such as acetaminophen, L-dopa, tolazamide, and ascorbic acid (Giordano et al., 1989).

GDH utilizes either pyrroquinolinequinone (PQQ), or nicotinamide-adenine dinucleotide (NAD) as cofactors (Bartlett et al. 2002). GDH is oxygen independent, however the cofactors are expensive and unstable. The mediators used in this system are ferrocene derivatives: ferricyanide, and quinones (Newman & Turner, 2005). These compounds have lower redox potentials avoiding interfering species and have high stability. Although the mediator reacts with an enzyme and does not use oxygen in its reaction, the possibility of dissolved oxygen competing with the mediator exists. The result of such a reaction leads to reducing efficiency and production of hydrogen peroxide. The mediator may also react with compounds in the blood, which would further compromise the accuracy.

GDH-PQQ can oxidize various mono and disaccharides such as mannose, maltose, and lactose (Tsujimura et al., 2006). Interference with other sugars in a blood sample when using GDH-PQQ as a biosensor may overestimate glucose concentrations. For patients receiving dialysis treatment, approximately 35% of diabetics, this biosensor would be totally contraindicated as infusion solutions for dialysis use high concentrations of maltose. GDH-NAD during glucose oxidation
becomes an efficient electron acceptor, the reduced form, NADH, can be electrochemically oxidized.

\[
\text{Glucose} + \text{PQQ (ox)} \rightarrow \text{gluconolactone} + \text{PQQ (red)}
\]

\[
\text{Glucose} + \text{NAD}^+ \rightarrow \text{gluconolactone} + \text{NADH}
\]

The electrodes inside the meter itself can cause measuring inaccuracies. Electrodes in the presence of electroactive species in blood cause interference in glucose measurements. Oxygen dependence on free oxygen as a catalytic mediator in the range of that of peroxide coincides with the oxidation potential of many other compounds found naturally occurring in blood. Frequent use of modified platinum electrodes become vulnerable to fouling by blood proteins and chloride ions (Toghill & Compton, 2010). The electrochemical oxidation of glucose is very complex. It involves adsorption, electron transfer, and chemical rearrangement. Platinum, which is considered the best material for the electrodes in these devices, is unable to provide adequate sensitivity to glucose (Vashist, et al., 2011). Poor selectivity and surface fouling affected by chloride ions, amino acids, creatinine, epinephrine, urea, ascorbic acid, and uric acid in the blood. These endogenous/exogenous compounds cause platinum to lose its sensitivity to glucose. The effect of other electroactive species and electrode fouling gives overestimations of the true glucose value. This overestimated glucose value can be life threatening should hypoglycemia actually be present and not recognized, or it is artificially induced by inappropriate insulin administration. Using the corrected glucose value by taking into account an individual’s hematocrit the true glucose value is obtained. Using paper microfluidics and fluorescence instead of redox reactions the only
component of interest is labeled and measured, the device will not have interfering species confounding the results. The test strips will not touch any of the internal parts of the measuring device therefor no biofouling, which could lead to errors. Each test strip will have its own positive and negative controls spotted o them, and the software for standard curves programmed into the device.

Conversion of Whole Blood Glucose Values to Plasma Values

Glucose meters measure whole blood not plasma samples as in a CL. Plasma numbers are generally 10-12% higher than whole blood (Joslin Diabetes Notes, 2015). It is not unusual for a glucose meter to be as much as 20% in error than a measurement performed in a CL (personal communication, and Saudek, C.D. et al. (2006). This is due to the fact that glucose concentrations are higher in the plasma than red blood cells. Anemia or polycythemia will cause significant variations in whole blood sugar values as the water content of plasma can be affected by these and many other disease conditions (Tustison et al, 1966). Some meters do translate whole blood measurements to plasma measurements. This conversion is based on the hematocrit. There is a difference between the amounts of water in plasma versus whole blood, so the glucose values are different due to water content. The International Federation of Clinical Chemistry (IFCC) set a conversion factor of 1.11 for plasma versus whole blood to correct for the difference in water content (personal communication, and Fogh-Anderson & D’Orazio, 1998). This number adds compromises to glucose quantitation as it assumes a hematocrit of 45% and a red blood cell to plasma water ratio of 0.76 (Rainey & Jatlow, 1995). For meters that do
not have this conversion option, the plasma glucose is estimated by adding 10% to
the whole blood glucose value. Attempts to convert glucose in whole blood to that in
plasma creates bias at the high and low hematocrit ranges, and both calculations
become invalid in people with hyperglycemia, dehydration, and/or hyperosmolality
among other health issues as many conditions will affect the hematocrit value, and
thus the glucose value. Morrison and Fleck (1973) found no simple numerical
relationship between plasma and whole blood glucose. If the assumption that
glucose is uniformly distributed in water of cells and plasma were true then, cell
glucose (E) would equal the cell water content (W) multiplied by the plasma glucose
(P). Using the derived equation \( B=(1-H) P + HWP \) by Morrison and Fleck, where
B=whole blood glucose, H=corrected hematocrit, they plotted \( B/P \) against \( H \) and
obtained a straight line. The correlation coefficient is however only 0.0752; it is so
low it is almost negligible. Ingram et al. (1971) compared the glucose values of
whole blood and plasma with the corresponding hematocrit of each sample pair and
found no relationship between these values and a correlation of zero. They propose
that for most purposes plasma glucose should replace glucose determination in
whole blood. Haeckel et al. (2002) rejects converting glucose concentrations to that
of plasma concentrations, and strongly recommended the use of plasma for all
glucose monitoring. Holtkamp et al. (1975) found that any conversion factors from
whole blood to plasma glucose may only be made when the samples are
deproteinized. The problem with converting whole blood to plasma values for
accurate glucose measurements is not simple; it may even be not possible. It
assumes a hematocrit of 45%, however in many patients this value will vary due to
other illnesses and metabolic states. This is why CL always uses plasma in calibrating their equipment, and most importantly in testing patient samples.

Using “filtered” whole blood, which is free from cellular components and proteins, glucose quantitation will be obtained without interference from oxygen as the sample is quickly wicked into the paper and not exposed to the environment.

Physical Factors

Physical factors that can affect the quantitation of glucose in a hand held devices are altitude, temperature and humidity (Giordano et al, 1989). Glucose test strips, particularly those using GOx, are very sensitive to oxygen concentrations. At high altitude GOx over estimates the glucose concentration by 6-15%. At higher altitudes increased production of red blood cells altering the hematocrit will affect the glucose value (Montagnana et al., 2009). However, GDH, which is not oxygen sensitive and is not as affected by oxygen change, reported glucose values within 5%. Temperature change is not predictable as it is environmental and cannot be easily controlled. Although glucose meters have sensors to report errors due to extreme temperature changes that affect the meter, but most importantly degrade enzymes, mediators, etc., results are brand-specific and not technologically dependent. Measuring glucose at 8°C reported errors between 5-7%, but the measurements could either be positive or negative. At elevated temperatures enzymes will denature and test strips will not function. In high humidity the enzyme can be rehydrated and render the protein only partially active (Tonyushkina & Nichols, 2009). The effects of altitude on the hematocrit are corrected by measuring
the hemoglobin. Converting the hemoglobin value to the hematocrit and thus correcting for a true glucose value. Increased temperature should not affect the device itself, as it is quite robust. However, test strips will be affected by elevated temperature and humidity due to degradation of reagents. This is to be expected for any reagents exposed to extremes in environment. Our device will alert patients that test strips have been compromised by having a spot of known concentration of reagent to be quantitated at the very end of the test strip. This will accomplish two things: 1) will tell the patient has strip inserted correctly by measuring the fluorescence output, 2) if the concentration of the reagent has gone down then the patient is alerted to use new strips. No SMBGs on the market have these error feedback warnings.

Is it realistic to have an error rate of 5% or less in glucose meters/test strips given the technology now on the market and no incentive to improve it? Vashist et al. (2011) states, “it seems that the technologies behind BGMD have already reached the advanced stage. There are still possibilities of incremental improvements but it will be highly difficult to come across a breakthrough technology that can further evolve.” Newman & Turner (2005) state “Meter companies have been very slow to introduce new technology and have relied on a strategy of putting existing technology in a new box, combined with a marketing campaign targeted at a specific segment of the market.”

The solution to this problem is an accurate and standardized POCGMD that does not suffer from the complications of the enzymes like the meters that are now on the market. The device should be accurate, precise and specific for glucose. It
should also include tests for hematocrit, ketones and glycated hemoglobin (A1C) (personal communication). Above all it should be technologically simple, and easy for patients to use. The device we propose will be extremely simple, user friendly, extremely robust for harsh conditions, and inexpensive enough that people worldwide can have their own device and not have to depend on infrequent visits from health care workers in remote areas or cell phones to relay each test result to central facilities for interpretation.

The glucose meter proposed here consists of measuring not only glucose levels, but would also include values for the hematocrit, ketones, and glycated hemoglobin. The hematocrit value greatly influences the glucose value; therefore incorporating it into glucose calculations will help remove additional confounders in glucose value calculations. Various illnesses and metabolic states affect the hematocrit and thus the true glucose value. Ketones are important to monitor for diabetic ketoacidosis, which can be fatal, and most patients don’t know they have this metabolic condition. The glycated hemoglobin (A1C) is a good measure of how well one’s glycemic control has been over a three-month period. If the A1C is in acceptable limits then treatment proceeds as usual, higher values indicate uncontrolled glucose levels and intervention is required. To try and maintain at least a 5% error in glucose measurements, or less, a fluorescent probe will be used and multiple readings averaged. Fluorescence is an extremely accurate and quantitative method. The glucose-probe complex will be measured 10 times, 1 millisecond apart and averaged. This complex is devoid of binding to exogenous and endogenous compounds as it is glucose specific. The hematocrit will be used to
calculate the true glucose value. The ketones will be measured via chemiluminescence, and red blood cells labeled with a fluorescent probe. The device itself will not become fouled from blood proteins or chloride ions, and the paper microfluidic device can be burned after use, thereby not producing a biohazard.

Glucose values are dependent on the hematocrit, therefore by incorporating the hematocrit into the glucose value a true glucose measurement can be obtained. Current meters assume a hematocrit of 45%, however various metabolic and disease conditions will affect this value and hence the true glucose concentration. By incorporating a hematocrit correction factor a true glucose value is obtained allowing the patient to self-treat appropriately and safely. Ketones must be incorporated as a test since ketoacidosis is a life threatening condition in diabetes mellitus. This metabolic condition can be present, and most times patients are unaware of the condition. Without immediate medical treatment morbidity and mortality are at stake. The A1C is a three-month window into how well one’s glycemic control has been. Although it does tell of one’s daily high and low glucose values, it also tells how well overall ones diabetes is being controlled. If the A1C is in the very low or very high range medical intervention is necessary. However, if there is good glycemic control and one’s A1C in an acceptable range then there is no adjustment in treatment, and morbidity and mortality decrease. With one finger stick all these values can be incorporated into one test strip and quantitatively analyzed via fluorescence.
The conversion hemoglobin to hematocrit is hemoglobin X .34 (personal communication). To convert hematocrit to the A1C the equation is (AG mg/dl=28.7 X A1C- 46.7, R^2=0.84, P<0.0001), AG=average glucose value (Nathan et al., 2008).

Ketones, β-hydroxybutyrate, the most common of all ketones can be measured, and a patient alerted if they are developing ketoacidosis, which could be life threatening. Increased ketone concentration in diabetics or in patients presenting with hyperglycemia suggest the possibility of diabetic ketoacidosis (DKA) (Hershman, Endocrine Pathophysiology: A Patient- Oriented Approach, second edition, chapter 8 Davidson, 1978). Ketoacidosis is a result of insulin deficiency, and decreases glucose uptake. Osmotic diuresis ensues and causes electrolyte depletion. This condition leads to increased production of ketone bodies, which exacerbates electrolyte depletion, most importantly overproduction of ketone bodies leads to acidosis. Patients with ketoacidosis don’t usually know they have this problem therefore it is imperative that they are able to check themselves.

Measuring the glycated hemoglobin (A1C) gives a physician a window into how well a patients' diabetes has been controlled over a three-month period. Although it is just a snap shot and doesn’t tell how many diabetic crises a patient has had in that time period, it is an important parameter. An A1C tells the physician that over a three-month period the patient’s glycemic control was either very tight or not well-controlled. The scale of A1C goes from 6, equivalent to 135mg/dl considered tight control up to 12, which is equivalent to 345mg/dl not controlled (Diabetes Care, 2003). The Diabetes Control and Complications Trial (DCCT) demonstrated that tight glycemic control, low A1C, reduced the risk of developing diabetic
complications (Gallagher, et al., 2009). Makris & Spanou (2011) confirm a close relationship between A1C and an average glucose value, although they suggest that different studies do yield different linear equations. This test would most likely be an approximation, and CL results will be used to corroborate the findings.

Fouling of the electrodes as discussed earlier is a problem and decrease testing performance. In a perfect device the testing strip should not touch any parts of the working device (Toghill & Compton 2010). The proposed device will work by fluorescent detection of glucose and red blood cells, and chemiluminescence of ketones. Glucose and DiL and DiO with their corresponding fluorophores will be stimulated at an appropriate wavelength with light emitting diodes (LEDs), and measuring the chemical reaction product of the chemiluminescent reaction of ketones using a charge-couple device (CCD) camera. The sampling will use paper microfluidics. No part of the testing system will touch any of the internal components, hence the potential of biofouling will be eliminated.

A USB port can be added for transferring of one's progress on a computer and then transmitting it to their physician. Date/time logs of when tests were performed and the results of all four parameters can be stored for two years or more for comparison of results and glucose maintenance. A mobile application (APP) would be very useful for self-monitoring or transmitting information to a physician. With the security of cloud-based information, medical information is safer than ever before.

Education however is the key to proper use and interpretation of all test results. Through hospital diabetic educational classes and working with one's
physician as a team, much better outcomes can be attained. The proposed device will offer much more than what is currently on the market, such as multiple tests with single finger stick, higher sensitivity, and higher accuracy.
Chapter II
Materials and Methods

Several detection and measurement techniques were used or adapted for developing the test devices presented in this study. In this chapter, we will describe the materials and techniques used to detect the glucose and ketone concentrations and to determine the red blood cell counts. We will also present some of the limitations of these approaches.

Detection of Glucose

A glucose/glucose oxidase kit using Amplex Red fluorophore is used (Molecular Probes)… The reaction used in this kit is glucose oxidase which reacts with glucose to form gluconolactone and hydrogen peroxide. Hydrogen peroxide is detected by the fluorophore, Amplex Red, excitation/emission is 571/585 nm. Generation of standard curves for glucose and hydrogen peroxide are as follows: concentrations and controls are: glucose concentrations from 0 to 100 μM. A 200 μM glucose solution will be the positive control for glucose, and the negative control is reaction buffer without glucose added. The positive control for hydrogen peroxide is a 10 μM solution; a negative control for hydrogen peroxide is the reaction buffer without H2O2. An Agilent Cary Eclipse Spectrophotometer will be used for this and all other protocols. The reacting reagents for glucose detection will be spotted on testing paper for detection via paper microfluidics using the spectrophotometer.
The paper microfluidics will have glucose as a positive control; a negative control will be mannose, and the blood sample. As a POC device it will average multiple readings and take into account the hematocrit for accuracy. Multiple readings nanoseconds apart can be averaged for more accurate concentration results. It will also have a positive, and negative control as well as the sample. None of the above are can be accomplished by meters presently on the market.

Detection of Ketones

To detect for ketones the enzyme $\beta$-hydroxybutyrate dehydrogenase is used. The main ketone $\beta$-hydroxybutyrate will be measured. A chemiluminescent detection kit from Sigma-Aldrich will be used. The concentration of $\beta$-hydroxybutyrate is determined by a coupled enzyme reaction which produces a colormetric product at 450nm. Generating the standard curve for $\beta$-hydroxybutyrate a stock solution of 1 mM $\beta$-hydroxybutyrate is prepared. In a 96-well plate 0(Blank), 4,8,12,16 and 20nmole/well, standards are added along with 50$\mu$L assay buffer. Calculations use the reading from the blank, which is subtracted from all readings as background. The paper microfluidics will have a positive control, $\beta$-hydroxybutyrate, a negative control will be methyl isobutyl ketone, and the blood sample. Although this is a chemical reaction and cannot average multiple values, it is a test that is extremely important in that it will warn a patient of ketoacidosis which most patients do not know they have, and is life threatening. Incorporating this into a POC device is necessary to avoid morbidity and mortality, and it adds another important test without an additional finger stick.
Determination of Red Blood Cell Count

Red blood cells are able to take up two different fluorophores, both of which are dialkylcarbocyanines, either 3,3'-Dioctadecyloxacarbocyanine perchlorate (DiO), or 1,1'-Dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiL). Stock solutions to determine the optimal concentration of dye needed to label red blood cells are: 0.01, 0.1, 0.25, 0.5, and 1.0 mg of dye /ml of solvent (Unthank et al. (1993). An aliquot of each solution will be added to RBCs and measured over a 30 minute period starting at time zero to 30 minutes, measured every 5 minutes, and the fluorescence observed for a maximum peak. The difference between them will be measured as to how completely the cells take up each dye, as well as how quickly. DiO excitation/emission in methanol is 484/501 nm; DiL excitation/emission in methanol is 550/567 nm. Multiple readings will be acquired and averaged. Obtaining the red blood cell count, hemoglobin concentration, will then be used to calculate the hematocrit for true glucose value corrections, and the A1C. The method used for this analysis is adapted from the research of Unthank et al. (1993). Calibration curves for all parameters:

a) glucose: glucose using Amplex Red Glucose/Glucose Oxidase Assay kit

b) ketones: chemiluminescent kit for quantitation of β-hydroxybutyrate

c) labeled red blood cells (personal donation) with Dil and DiO fluorophores

Using Beer’s Law, Absorbance= eLc, e=molar extinction coefficient, L=path length of the cell holder, and c =concentration of the solution, the concentration of a
solution is plotted versus the absorbance; this gives a linear relationship. The spectrophotometer will calculate and print the standard and experimental results.

Three types of paper will be used in a size exclusion manner (Millipore). The largest pore sized paper will retain the cellular blood components, a smaller pore sized paper will retain the proteins, and the smallest pore sized paper will retain the “water” portion of the whole blood, which contains the smaller molecules such as glucose and ketones. Either lateral flow, or wax imprinting can be used to form testing areas where the cells and molecules of interest are to be detected. Reagents, which specifically bind the components of interest as well as control areas, will be placed onto the paper using a pipette, and dried. At the very end of each test strip a fluorophore will be placed, which indicates to the charged couple device (CCD) camera (via programming the device in language C) that the strip is fully saturated and testing can proceed. The size of the testing paper will be calculated using the Washburn equation: \( L^2 = \frac{\gamma D t}{4 \mu} \), \( L \) is the distance moved by the fluid front, \( t \) is the time, \( D \) is the average pore diameter of the paper, \( \gamma \) is the surface tension which includes the contact angle, and \( \mu \) is the viscosity of the fluid. Viscous resistance, which is proportional to the velocity of flow, is \( 8 \mu L / D^2 \), this will increase as the length of the paper increases (Fu et al, 2011).

Polymethylmethacrylate (PMMA) sheets will be laser cut to fit the size of the paper microfluidic test strips; the paper will be sealed inside for protection from the environment and for ease of handling. There will be cut outs on the top cover of the PMMA for sample deposit and light detection where reactions occur. A conceptual drawing of such a device is shown in Figure 1.
Figure 1. Conceptual drawing of a paper microfluidic device. This drawing demonstrates the cross-section of the laminated paper microfluidic device concept and shows the individual components.

Limitations

One of the limitations of this method is that the volume of blood needed for lateral flow paper microfluidics surpasses that of test strips now on the market. Using a vertical layered system versus lateral system may be needed to decrease the blood volume needed for testing. Non-specific binding of the negative controls with the fluorophores could be a possibility. The length of time for the reaction of fluorophore with control reagents and samples is excessive. Stability of fluorophores and enzymes spotted and dried on paper can be affected by the length of time they “sit” on the paper, temperature, and humidity. Fluorophores for glucose and red blood cells can be measured multiple times, however, the draw back to measuring ketones could be an issue. Chemiluminescence is a chemical reaction and can only occur once limiting its measurement to one time point. Due to lack of funding using a fluorophore is not possible at this time. AAT Bio in California has a fluorescent dye kit for β-hydroxybutyrate with an excitation/emission at 540/590.
nm. Using a fluorophore in the future would allow multiple readings for averaging and reporting a more accurate ketone concentration. Labeling for ketones, and red blood cells doesn’t work and further calculations can’t be achieved. The error for glucose measurement is 5% or higher, and doesn’t achieve a better outcome than glucose meters now in the market place.
Chapter III
Results

Standard curves were generated for the chemiluminescence reaction for ketones, glucose and glucose oxidase. These curves for ketones, glucose and glucose oxidase are presented in Appendix 1, Appendix 2 and Appendix 3, respectively. The paper microfluidic detection portion of this work for ketones, glucose and red blood cells could not be performed due to the fact that the Cary Eclipse spectrophotometer did not have the appropriate holder for measurements for solid materials. We used a SpectraMax M2 spectrophotometer because the Cary Eclipse spectrophotometer was in use by another researcher. This spectrophotometer did not calculate nor print out the graphs for the standard curves.

The chemiluminescence reaction for ketones was carried out per the protocol with the kit. β-hydroxybutyrate assay buffer, β-hydroxybutyrate enzyme mix, and β-hydroxybutyrate substrate mix were added to all wells except the first well where the enzyme mix was not added, and served as the blank. The concentrations of β-hydroxybutyrate per well except the blank were 4, 8, 12, 16 and 20 nmol/well. The absorbance was measured at 450nm for all wells; the blank was then subtracted from all other readings. We were unable to incubate the well plate for 30 minutes at 37°C due to the lack of an incubator in the laboratory we were working in. The correlation coefficient (r) for our standard curve was r=0.847.
The fluorescence measurements of glucose and glucose oxidase were performed per the protocol with the kit. The Amplex red glucose/glucose oxidase assay kit provided a one-step method for the detection of either glucose or glucose oxidase. The excitation/emission for the reaction products is 571/585nm. We were unable to incubate the well plate at 37°C for 30 minutes per the instructions because the laboratory we were working in did not have an incubator. The Amplex Red reagent is air sensitive and needed to be protected from light. However this was not possible due to less than ideal conditions in the laboratory we were able to work in. Glucose concentrations were 0.25, 50, 75, and 100 μM, 0μM served as the blank and was subtracted from the rest of the values. Glucose oxidase concentrations were 0, 2, 4, 6, 8, and 10 mU/ml, 0mU/ml served as the blank and was subtracted from all other values. The correlation coefficient (r) for the glucose standard curve was r=0.759 and for glucose oxidase r=0.895.

Based on the paper properties from Millipore, the bed volumes of the paper membranes were extremely high. The porosity values of the papers are the portion of volume that is air. The volume of liquid in the pores per area can be calculated given the thickness. The bed volumes, the volume that are pores, are calculated by multiplying the total volume by the porosity. The bed volume is the amount of liquid that the paper holds when saturated. Millipore supplied the bed volumes to us. The papers with the smallest bed volumes were used in a layered fashion calculating the Washburn equation in the Y direction, not the X direction. We decided this was a better approach than lateral flow in the X direction because it would be faster and require a smaller volume of sample. Four papers were selected to use in this layered
fashion, paper membranes HVPP, DVPP, BVPP, and HEMF. The papers are hydrophilic membranes made of polyvinylidene fluoride (PVDF), attributes of the individual papers are:

1) HVPP: 0.45μm pore size, 115μm thick, flow rate 29ml/min/cm^2, bed volume 6.9ml
2) DVPP: 0.65μm pore size, 115 thick, flow rate 69ml/min/cm^2, bed volume 6.7ml
3) BVPP: 1.0μm pore size, 115μm thick, 87ml/min/cm^2, bed volume 10.1 ml
4) HEMF: 0.45μm pore size, 142μm thick, 24ml/min/cm^2, bed volume 11ml

DVPP was used as the top layer, HVPP middle layer, and BVPP as the bottom layer in a size exclusion manner, going from smallest to largest bed volume. The larger particles should pass through to the bottom layer, and retain the smaller molecules on the top two layers. Using another layered approach where the larger particles are retained on the top layer and smaller particles pass through to the bottom layer was also performed. Another configuration using DVPP, HVPP, and HEMF were used in a layered manner, going from the largest bed volume to the smallest bed volume. The fabrication of the three layered microfluidic devices proved challenging. We hand drew hydrophobic barriers using a sharpie pen which contains hydrophobic ink to contain the liquid sample. This was adapted from method found on YouTube by Dr. Sangeeta Bhatia in which she coated entire papers with hydrophobic ink except for the areas where channels were drawn. This allowed the liquid to penetrate through multiple layers without “bleeding” outside of the testing areas. We tried to imitate this by using only a sharpie, just to outline the testing areas, however there was “bleeding” outside of these areas. Initially food
color was used to see if the fluid was retained in the areas where the ink was used to pattern the testing areas and if the sample penetrated through the three layers.

Figure 2 presents a picture of a 3D layer with hydrophobic inked channels and food coloring simulating blood sample.

Figure 2. 3D microfluidic device. In this picture, the red food coloring simulating blood is seen to penetrate through the three layers of the device. 10g weights were used to press the layers against each other for good contact.

We first adhered the papers together with double-sided sticky tape, however the paper layers were not in contact with each other due to the thickness of the tape. We placed two 10g weights on the top paper layer and found that it allowed the papers to contact one another.
We decided to laminate all further paper devices for optimum contact. Sheets 3mils thick were used to laminate the devices and inlet holes were cut into the sheets above the testing areas using an X-Acto knife and small scissors prior to lamination. Figure 3 shows multiple paper microfluidic devices laminated between two Mylar sheets.

![Figure 3. Paper/Mylar microfluidic devices. Paper layers were laminated between two Mylar sheets for proper sealing of the devices. Here four separate devices are seen.](image)

Confining the liquid to the test spots and channels even after laminating still proved to be an issue. There was some bleeding of the ink so a red crayon was used to outline these areas much like wax imprinting. It must be pointed out that the
paper membranes are very fragile and any pressure does puncture and/or rip them. Great care was used to apply the “wax” from the crayon but it didn’t measure up to the precision of a wax printer. There was no consistency over the control of size of the test areas as they were drawn by hand not machine printed. Another problem was the inability to layer the membranes such that every testing area precisely overlapped; this could have been the problem with the samples not penetrating to the bottom layer. Additional holes at the outlets to the channels were not made which created a slower flow rate by trapping air.

We decided to discontinue using food color due to its bleeding around and under the paper membranes. Finger sticks for blood were made and applied to four different microfluidic devices. The layered devices using DVPP, HVPP, and HEMF was the best configuration and had the highest flow rate, the channels on the top layer completely filled within 18 minutes, however had outlet holes had been made releasing trapped air the flow rate would have been faster. The DVPP, HVPP, and BVPP device also filled within the same time frame, however the middle channel did not fill completely this could be due to the difference of the channel size (Figure 4).

The two configurations using HEMF, BVPP and DVPP or HEMF, BVPP and HVPP showed a pink “halo” of fluid around the test spot on the top layer, this demonstrated the ability to separate the cellular portion of the blood from the plasma (Figure 5). There was no sample penetration through the third layer on any of the paper microfluidic devices.
Figure 4. Filling of devices with whole blood. In this picture, whole blood is seen filling the three arms of the laminated paper devices through the opening in the top (Mylar) layer, where the drops of blood can be seen at the intersection of the three arms of each device.
Figure 5. Separation of blood cells from plasma on a device. The slightly pink halo seen around the drop of blood at the inlet of this device is the plasma being separated from the blood cells via the filtration effect of the paper layer.
Using chemiluminescence for ketones was inconclusive as we were not able to measure the reaction with a true sample. A major drawback to this is the thirty-minute incubation period, which is clinically unrealistic in that patients aren’t going to wait that long for a test result.

One of the drawbacks to using the Amplex Red assay kit is at extremely high levels of glucose (500μM) it can produce lower fluorescence than a 100μM concentration due to excess H2O2 generated from the reaction of glucose with glucose oxidase, which can then oxidize the reaction product, resorufin, to a nonfluorescent product resazurin. There is also a problem with detection of glucose oxidase. At very high concentrations of glucose oxidase (50mU/ml) a lower fluorescence response is detected than with lower levels (10mU/ml). This is due to excess H2O2 resulting from the reaction of glucose with glucose oxidase, which can then oxidize the reaction product, resorufin to nonfluorescent resazurin. Given these two drawbacks along with light sensitivity and reaction to air, it would not be suitable as a detection reagent for glucose in a paper membrane microfluidic device. The reaction to air is not surprising as it is a similar problem seen in commercial test strips that use glucose oxidase as an enzyme, and was discussed in detail in the body of this work. This test is also inconclusive, as we could not measure the
fluorescence of a real sample. As with the ketone detection, a thirty-minute incubation period is required and clinically this is unacceptable.

Due to the large bed volumes a lateral flow paper device would require too much blood volume, and increase in the volume of blood needed, this translates into the depth of lancet penetration to obtain the sample, and increased pain to the patient. We decided a layered approach would require a smaller blood volume and faster transit through the paper as oppose to a lateral flow process that would perhaps require more time. The layered approach can be tricky, however very small test spots and channels can be made with a laser cutter, wax imprinting or using hydrophobic ink to cover the entire membrane surface except test areas per the method by Bhatia, and lamination would produce a very good test strip. Using a laser cutter, wax printer and laminator we would be able to precisely control the sizes of testing areas and seal the paper devices with inlets and outlets using a laminator. We were not successful with our samples penetrating the three layers of paper membranes. This could be due to higher bed volumes and not enough sample to penetrate. Another explanation is in overlapping the papers, test spots and channels might not have been precisely overlapped and the ink and/or wax interfered with the sample migrating through the membranes. However, we were successful in separating the blood cells from the plasma. This was evident from the halo of plasma around the blood cells. This was proof of concept that a three-dimensional paper membrane device will work when the appropriate equipment is available and fabrication can be performed accurately.
In the future under stringent laboratory conditions this project can be revisited, and chemiluminescence and fluorescence measurements can be taken at 5-minute intervals to determine how long the incubation period has to be in order to get a precise reading in less than 30-minutes. Utilizing a laser cutter, wax printer, and laminator will allow consistent testing areas, which will be much smaller than what had been hand drawn. The paper membranes can be precisely layered so sample penetration through the three layers is obtained. The test site for glucose and ketones will be spotted via an ink jet printer using appropriate positive and negative controls. For glucose the negative control will be mannose, the positive control will be glucose. For ketones the negative control will be methyl isobutyl ketone, which is similar to β-hydroxybutyrate but will not interfere with the substrate to be detected, and the positive control will be β-hydroxybutyrate. This will be the top layer of the paper microfluidic device using differing bed volumes. Testing for red blood cells using either DiL or DiO will be on the bottom layer. To check for protein retention and separation by the papers ninhydrin can be used to stain the individual layers and see where the proteins are retained. It is possible that all three layers may have some proteins due to the varying sizes of proteins. It may be impossible to completely separate out of the proteins using this technique; however, the vast majority of proteins should be separated into one layer.

Proof of concept has been shown for this first generation microfluidic device by the separation of plasma from blood cells. The issues to solve for a second-generation microfluidic device will incorporate the method by Bhatia or some aspects of fabrication by the Whitesides group (Martinez, et al, 2008). The
Whitesides group fabricated their three layered devices by stacking layers of paper and water-impermeable double-sided tape in alternating layers, both patterned in such a way that channels the flow of fluid within and between these layers. A hydrophobic polymer was also used and patterned into the paper outlining the channels through which the fluid flows laterally. The double-sided tape separates the channels vertically, and holes cut into the tape allow the vertical flow of fluids.

Utilizing a laser cutter, wax printer and laminator along with a combination of the two above methods, or a better approach still not realized we believe a microfluidic device will be successful. Analyzing the reagents that detect ketones, glucose, and red blood cells under stringent laboratory conditions, and the ability to measure the absorbance of the fluorophores on the paper membranes using a spectrophotometer equipped with a material holder will yield information as to the feasibility of using this detection method.
Appendix 1

Standard Curve for Ketones

Absorbance vs Concentration of β-hydroxybutyrate (nmol/well)

Correlation coefficient (r): 0.847
Sample size: 6 β-hydroxybutyrate nmol/well

Mean x (\(\bar{x}\)): 10

Mean y (\(\bar{y}\)): 145.77666666667

Intercept (a): 75.189523809524

Slope (b): 7.0587142857143

Regression line equation: \(y=75.189523809524+7.0587142857143x\)
Appendix 2
Standard Curve for Glucose

Absorbance vs Concentration of glucose (μM)

Correlation coefficient (r): 0.759
Sample size: 5  Glucose (μM)

Mean x (\(\bar{x}\)): 50

Mean y (\(\bar{y}\)): 1393.29

Intercept (a): -1003.44

Slope (b): 47.9346

Regression line equation: \(y=47.9346x-1003.44\)
Appendix 3

Standard Curve for Glucose Oxidase

Absorbance vs Concentration of glucose oxidase (mU/mL)

Correlation coefficient (r): 0.895
Sample size: 6  Glucose oxidase (mU/ml)

Mean x (\(\bar{x}\)): 5

Mean y (\(\bar{y}\)): 9959.3666666667

Intercept (a): -5780.4476190476

Slope (b): 3147.9628571429

Regression line equation: \(y=3147.9628571429x-5780.4476190476\)
References

*International Diabetes Federation Diabetes Atlas, 2014*
- Sixth edition Update, International Diabetes Federation, 2014

- Review of electrochemical sensors and their applications in diabetes management
- Discusses diabetes with regard to glucose meters

- Review of glucose meters and technical challenges
- Discusses glucose detection, meter performance criteria, clinical accuracy, potential interferences in glucose measurement
- Effects of elevated temperature on test strip enzymes

- A history of glucose biosensors and overview of use in clinical practice
- Discusses challenges related to achievement of accurate and reliable glucose monitoring
- Technical improvements in glucose biosensors, and standardization of analytical performance

- Assessment of glucose meter specifications by modeling of errors in insulin doses
- Proposed specifications of glucose meters allow results to be in error by 5-10% or more of true glucose value
- Aimed to characterize the quantitative effect of meter error on the ability to find the correct insulin dose for the true glucose value
● Used Monte Carlo method to provide the intended insulin dosage 95% of the time required glucose meter be <1% or <2%.

● A commercial prospective on home blood glucose biosensors
● Emphasis on commercial developments in home glucose testing market

● A perspective and evaluation of electrochemical non-enzymatic glucose sensors
● Addresses merits and shortfalls of non-enzymatic electrochemical glucose sensors with respect to their commercially available enzymatic counterparts
● Biofouling of platinum electrodes by blood proteins and chloride in blood

Joslin Diabetes Center; Newsletter, 2016
● Benefits and disadvantages of continuous glucose monitors

● A review focusing on point-of-care (POC) glucose meters and the accuracy of the measurements

● Sources of errors in glucose measurements
● Factors affecting blood glucose meters

● Compared several point-of-care glucose monitoring devices (POCGMD) against a reference method
● Found out of several devices tested only three had acceptable accuracy
● Physical factor affecting SMGDs

• Blindly evaluated four POCGMDs; found none achieved the 1996 ADA requirement for accuracy


• Tested twenty-four POCGMDs; found more than 40% did not meet minimum accuracy requirements of the German Institute for Standardization of 2003

*Joslin Diabetes Notes*, 2015

• Discuss whole blood versus plasma glucose measurements
• Plasma values generally 10-12% higher than in whole blood


• Discuss the significance of plasma glucose values
• Necessary to establish new standards for interpretation of plasma glucose levels


• Discusses confounders to accurate glucose measurements
• Whole blood versus plasma measurements
• Importance of hematocrit value


• Compared whole blood and plasma glucose values
• Found no conversion factor was can be derived from whole blood to plasma glucose values


• Tried to find a conversion factor for measuring whole blood versus glucose values
• These values should be predictable from the hematocrit, found no such relationship
● Discusses experimental data which indicates only the use of plasma samples be used for glucose determination

● A review article discussing the technology of commercial blood glucose monitoring
● Future trends in blood glucose monitoring
● Drawbacks to platinum electrodes in SMGDs

● Discusses the market for electrochemical biosensors
● Electrochemical techniques for measuring different analytes, manufacturing of single use disposable tests

● Effects of oxygen on glucose measurements using enzymes
● Glucose oxidase and glucose dehydrogenase

● A novel FAD-GDH and its enzymatic properties are discussed
● Insensitive to oxygen
● Can also oxidize other sugars as well as glucose

● An overview on blood glucose self-monitoring
● Altitude effects on oxygen concentration and hematocrit
- Conversion of hematocrit to A1C

- Textbook of medical endocrinology
- Chapter on ketones and ketoacidosis

- Diabetes Control and Complications Trial demonstrated low A1C reduced diabetes morbidity

- Confirm close relationship between A1C and average glucose value
- Discussion of paper microfluidics
- Washburn equation
- Viscous resistance

- Research article on labeling red blood cells for microvascular measurements
- Used two fluorophores, Dil and DiO, in vitro and in vivo to determine the correct concentration for maximum fluorescence in red blood cells

- Review article of methods, standardization, confounders, clinical value and controversies/recommendations for use of SMBGs
- Study of benefits to SMBG to A1C values and glycemic control
- Recommendations for use of SMBGs
- Discussion of the value of A1C levels
- Direct reading biosensors measure molality (glucose per unit water mass) in a sample
- Measuring molality via direct measure yields more quantitative results in blood and plasma than by measurements of concentration, ratio of water concentrations in calibrator and sample

- Article describes fabrication of three-dimensional microfluidic devices by stacking layers of patterned paper with double-sided sticky tape
- This configuration uses techniques difficult to duplicate in other fabrication methods
- The devices wick and distribute microliter volumes of fluids from a single inlet into arrays of multiple detection zones