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A Disposable Microfluidic Cartridge for Point-of-Care Blood Analysis

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By

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Abstract

Point of Care (POC) devices optimize medical care by providing quick diagnostic information to healthcare providers, enabling them to make critical treatment decisions under limited time constraints. A POC blood analysis diagnostic device through the means of a low cost disposable microfluidic cartridge could analyze accurate and fast test results at the patient bedside. The microfluidic cartridge would limit biohazard exposure, maintain sample integrity, and remain affordable. The biocompatible acrylic cartridge was run through a series of testing protocols including proper sealing, dynamic flow, vertical orientation, maintaining sample integrity, and temperature control. The design and test use a syringe pump system as a blood flow method. The cartridge sealed properly and worked in different handheld orientations without leaking fluid. It did not effectively maintain a certain temperature. While using rice starch as a blood substitute, the syringe pump was able to maintain the starch integrity while pushing the solution through the cartridge channels. This project needs testing with blood samples to further verify the cartridge.

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List of Acronyms

AFM – Atomic Force Microscopy
CFD – Computational Fluid Dynamics
ED – Emergency Department
FMECA – Failure Mode and Effects Analysis
GC – Gas Chromatograph
HPLC – High-Pressure Liquid Chromatography
ICU – Intensive Care Unit
IEC – International Electrotechnical Commission
IL – Instrumentation Laboratory
LCD – Liquid Crystal Display
LED – Light Emitting Diode
LOC – Lab on a Chip
MEMs – Microelectromechanical Systems
MQP – Major Qualifying Project
NICU – Neonatal Intensive Care Unit
PANi – Polyaniline
PDMS – Polydimethylsiloxane
PEEK – Polyether ether keyton
PMMA - Polymethylmethacrylate
POC – Point of Care
POCT – Point of Care Testing
PVA – Polyvinyl alcohol
RBC – Red Blood Cells
RFID – Radio – Frequency Identification
RGB – Red Green Blue (color saturation levels)
RoHS – Restriction of Hazardous Substances
RPM – Rotations per Minute
WBC – White Blood Cells
μTAS – Micrototal Analysis Systems

Executive Summary

For our Major Qualifying Project, we collaborated with Instrumentation Laboratory (IL) to create a disposable cartridge that can perform blood analysis on the electrolyte concentrations of Na^+, K^+, and Cl^-, and pressures of O_2 and CO_2. This project focused on creating a Point-of-Care (POC) medical device which would utilize a microfluidic cartridge. Microfluidic cartridges hold small amounts of fluid for transportation, metering, mixing, and other processes with the potential of minimizing the amount of blood needed with each blood analysis test. The microfluidic channels can be designed to maintain laminar fluid flow and preserve the integrity of the blood sample. POC devices use these cartridges because they are small and can be used at the patient's bedside, and thus optimize medical care by providing quick diagnostic information such as blood gas values to healthcare providers. With POC devices performing at the patient's bedside, critical medical decisions can be made immediately without the time delay of waiting for laboratory testing results.

The goal of this project was to design and develop a disposable, microfluidic cartridge to be used in a handheld blood analyzer for POC use. We aimed to achieve this goal through four objectives: reduce sample volume, simplify fluidic control, maintain sample integrity, and increase portability. Reducing sample volumes allows for less blood in the system which makes it easier for the user and moves the blood quicker. Simplifying fluidic movement control through microfluidic movement should make positioning the fluid over sensors easier and the device more portable without harming the blood sample integrity. To maintain sample integrity, the cartridge should avoid blood clotting and blood lysing. Finally, increasing the portability of the POC device makes it more user friendly, more affordable, and versatile in multiple medical environments. We followed these objectives as the project developed.

IL also gave us 18 design requirements for the device that fall into three categories: disposable cartridge, blood analysis, and operator interaction. The requirements called for a user friendly device with an industry standard compliant, lost cost, and safe disposable cartridge that could perform quick and accurate blood analysis on a small amount of blood. Therefore, we extrapolated four components that we worked on to achieve the design requirements. The first focused on a method of fluid movement that could control the flow of blood and its positioning. The second was the microfluidic cartridge that would take in blood from a syringe. Next, there was a microcontroller that would read the sensor data from the cartridge as well as control the method of fluid movement if needed. Finally, there would be a user interface that would display the system status to the user through the microcontroller.

The first step in our design process was to choose a fluid movement method based on its reliability on repeatability to create accurate results with each use. We also looked at the points of failures for each alternative design that would have compromised the safety of the user. The most important process of choosing a final design was performing a concept ranking using the 18 design requirements using a Pugh Matrix. There were six designs: a differential pressure design involves creating a cartridge with negative pressure in the channels, a compressed air design also used negative pressure but outside the cartridge channels rather than inside, a microneedle array is a patch of needles that uses capillary action to pull blood directly from the patient, and three active pump designs - a peristaltic pump, solenoid pump, and syringe pump. Based on the values of the Pugh Matrix ranking,

the top two designs selected were the syringe pump and the peristaltic pump. However, due to the time and manufacturability limitations of the team, the syringe pump was chosen as the fluid movement method.

With the syringe pump, there were two mechanical options that would allow the motor to interface with the syringe plunger itself: rotating gears and pulling plates. With the rotating gear design, there are more points of contact and error points such as the occasional skipping of gear teeth. The plate pulling system allowed for a linear relation between the rotational turning rate and the volumetric flow rate, making it easier for us to control the syringe pump. The plates are also a more compact system than the gears which will fit better in the handheld device. Therefore, we chose the plate pulling system for the syringe pump.

For our microfluidic cartridge component, we created the design in SOLIDWORKS with a top and bottom plate that would be adhered together using epoxy. The cartridge is made out of clear, biocompatible PMMA and was manufactured through micro-milling the plate cuts. Assembled together, the channel diameter is 1 mm with the exception of the inlets that have a smaller diameter to interface with a 22 gauge syringe. We left markers along the floor of the channels for future sensor placement. Due to issues with epoxy seeping into the channels, we redesigned the channels to have a tongue and groove design along the channels. Additionally, protrusions on the corners of the chip allows for accurate placement and alignment of the two plates without letting epoxy seep into the channels.

For our open source programming microcontroller, we chose an Arduino UNO board that could control the motor for our syringe pump. It also could be used to interface with the sensors once they get implemented into the cartridge. The Arduino board also controlled to LEDs and LCD screen, both are methods for user interface and displaying the system status to the user. This addressed all the components that we wanted in our design.

The next step in our project was the design verification step using multiple protocols we created. Due to the restrictions on acquiring blood samples for testing, we needed to find blood substitute that is easy to obtain and has similar mechanical properties to blood cells. We chose to explore rice starch mixed with water as a substitute solution. Therefore, the first verification process was to verify that rice starch with water has similar properties to blood in grain size and viscosity. These protocols called for measuring the size of the rice starch under a microscope and using a viscometer to measure the viscosity of different ratios of rice starch in water. After data analysis, we verified that rice starch was close enough in size to red blood cells to use in preliminary testing.

The other protocols test for biohazard exposure from the cartridge, temperature maintenance, fluid movement, and sample integrity. We wanted to limit biohazard exposure by ensuring that the cartridge did not leak fluid between the two plates after applying the epoxy. After pushing dyed solution through the channels of the cartridge, we measured the Red Green Blue (RGB) color values to see if there are any changes. We also rotated the cartridge with fluid in it along the X and Y axis to make sure it can turn in different orientations without leaking out of either the inlet or outlet. It was concluded that the cartridge does not leak and it limits biohazard exposure from the user.

Another design requirements was to maintain a fluid temperature of 37°C while the sensors were reading, and therefore we wanted to verify that the design is capable of doing so. After placing the cartridge on a heating plate of different temperatures, we would use a 66 gauge thermocouple to

measure the temperature in the channel once fluid had been pass through over time. We determined that the plate is able to heat up to that temperature within the 3 minute requirement.

We modeled the fluid flow through the cartridge to make sure there were no high pressures or high velocities through the channels that would harm the sample's integrity. We determined that a flow rate of 30 μL/sec was fast enough to position the blood over the sensors in a timely manner without affecting the sample's integrity. Then we had to convert that rate to the rpm of the motor for the syringe pump. Then, we check the sample integrity of the rice starch by running it through the cartridge using a benchtop syringe pump and our designed syringe pump. Both worked at the 30 μL/sec rate without critically damaging the rice starch grains.

After running all of our design validation tests, we confirmed that our design met 14 of the 18 design requirements given. The ones we were not able to confirm were:

- Complete analysis on whole blood
- Maintain blood sample integrity.
- Fit into the palm of the users hand
- Interface with the device electronics to support sensor functions

With the first two requirements, we would need to confirm by testing with whole blood samples to further verify our device beyond just using rice starch and water as our test sample. Our project currently does not fit into the palm of the users hand well for testing purposes. However, all the device components can be minimized in size to be more compact. Finally the next big step in the project will be to add the sensors to the cartridge and interface the cartridge with the electronic devices.

With this project, we created a microfluidic blood movement device. We created a disposable microfluidic cartridge and a syringe pump that can pull blood into the cartridge. Our Arduino microcontroller can control the stepper motor and relay the system status the user. Based on our testing protocols, our design was able to fit most of the design requirements given to us by IL at the beginning of the project. We achieved our objectives of reducing blood sample volume, simplifying fluid movement control, maintaining blood sample integrity, and increasing the portability of the device. The next step of this project is to implement the blood analysis by incorporating the sensors for blood gas analysis and interfacing the sensors with the microcontroller for data analysis. We were able to design the preliminary design of this POC blood analysis device for this project, which we hope will help healthcare providers make critical diagnosis in a variety of situations and improve the quality of patient care.

1. Introduction

Microfluidic science studies and applies the flow of liquids inside micrometer sized channels. "Microfluidic devices offer automatic and high-throughput screening, and operate at low volumes of consumables…in medical, veterinary, and environmental sciences" [3]. Biomedical engineering has merged with over two decades of microfluidic research and development to give rise to new applications such as genetic analysis, cell culture platforms, biosensors, pathogen detection systems, point-of-care (POC) diagnostic devices, and targeted drug delivery. Future improvements involve better interactions with macroscale laboratory processes and incorporating all the possible functions in one microfluidic handheld device for more usability [4]. Microfluidic technology has promising applications as the field continues to develop with their compact size and diverse functionality [4].

Point of care testing (POCT) is quickly allowing traditional laboratory medical testing to be performed closer and closer to the patient, allowing for medical decisions and care to be made faster. One such innovation in diagnostic care is a blood gas analyzer. Blood gas values, which include Na^+, K^+, and Cl^- electrolyte values, as well as partial pressures of O_2 and CO_2, are common indicators of many potential medical conditions, such has heart or kidney failure, diabetes, chemical poisoning, or drug overdose. They have become a mainstay in modern medicine, and their availability in the hospital setting rather than clinical laboratory has not only shortened diagnosis time, but also saved lives.

Microfluidic cartridges solve the "need for miniaturized, low-cost or disposable biosensors capable of rapid detection and accurate identification" in biomedical applications [5]. However, the development of one cartridge requires expertise across several disciplines ranging from fluid mechanics on the micro level, hemodynamics, and biocompatible materials. Microfluidic devices are economically cheap, disposable, and can be made into handheld devices. The ability to measure and analyze small volumes of fluids allows for deeper understanding and advancement of the healthcare sciences and engineering, providing quick and accurate results at the patient's side. Microfluidic devices are a step in that direction, taking biomedical applications into the micron level.

Our project focused on integrating the existing electrolyte sensor technology of Instrumentation Laboratory (IL) into a hand held device using microfluidic technology capable of performing venous and capillary blood gas analysis in the hospital setting, specifically in emergency rooms. IL currently does not have a handheld model of their blood gas analyzers. The combination of ILs patented technology in conjunction with the portability of our proposed device would allow for accurate and immediate blood test results at the patient bedside.

Instrumentation Laboratory gave us a list of project requirements that the design must satisfy. The design requirements umbrella over three main parts: disposable cartridge, blood analysis, and operator interaction. This balance of all three areas have been carefully coordinated to create not only a functioning blood gas analyzer, but one worthy of clinical use in comparison with market competitors. Keeping those requirements in mind, we performed in depth research on different types of microfluidic technology. Many materials, orientations, and regulations were found that were potential contributors to our final design such as microneedles and microfluidic channels. Interviews were conducted with graduate students as well as healthcare professionals in order to evaluate the best method of creating and designing our device to ensure good user interface. Next, the team came together to produce preliminary designs stemming from the literature review. We then collaborated with IL to finalize and distill these designs to narrow down the selection.

The final design consist of a biocompatible, acrylic cartridge with microfluidic channels and placement holes for the sensors. The fluid movement is controlled by a syringe pump consisting of a stepper motor and plate pulling system to pull the fluid into the microfluidic channels. An Arduino board controls the volumetric flow rate as well as conveys the system status to the user. This final design was able to comply with the three main design requirements: the cartridge as designed to be cheaply disposable yet easy to integrate with IL's current sensor technology. The Arduino board provided feedback to the user concerning testing progress via an LCD screen and LED, while the handheld design of the analyzer allows for comfortable use.

To verify the design, the microfluidic cartridges were put through a series of tests to ensure compliance with each of the specific user requirements. A rice starch solution was used as a blood substitute ready availability and also its particle size resembling that of RBCs. These tests include cartridge sealing, handheld orientation, and vertical angel testing protocols to confirm that no biohazardous leaks occur in the cartridge while in use. Dynamic flow tests determined the different volumetric flow rates through the channels while stationary flow tests showed the positioning capabilities of the system regarding proper fluid-sensor placement. The sample integrity, contamination, and temperature tests allowed us to see that the rice starch sample was not lysed or contaminated, yet capable of maintaining set temperatures consistent for accurate testing.

Our goals were to increase portability, reduce sample volume, and simplify fluidic control. Our final design and testing proved to satisfy most of the user requirements, including complying with industry and safety standards for medical devices. The microfluidic cartridge proved to be a safe, small, cost-effective and disposable for a handheld blood analysis device. The Arduino board and syringe pump interface with the microfluidic cartridge and each other well while still complying with the user requirements. However this design needs further verification to be complete. The testing needs to be completed with blood samples to ensure blood sample integrity while being pulled through the microfluidic cartridge. The system also needs to have an interface between the sensors, data analysis system, and the display screen on the handheld device. Although the microfluidic cartridge, syringe pump, and Arduino board completed a majority of the user requirements such as providing stable fluid movement as well as an ideal temperature and sterile environment for testing, there are still more steps to further complete this disposable microfluidic cartridge for POC blood analysis.

2. Literature Review

2.1 Microfluidics

Microfluidic cartridges or lab-on-a-chip (LOC) were developed in the 1950s in an effort of dispensing small amounts of liquids in nano- and sub-nanolitre ranges in order to build ink-jet technology that is available today [6]. By the 1960s, photolithography were developed that enabled the size minimization of thousands of transistors on semiconductor wafers that allowed for the first microprocessors [7]. It evolved to implementing a miniaturized gas chromatograph (GC) on a silicon wafer in 1979 and later, to the first high-pressure liquid chromatography (HPLC) in late 1980s [6]. For the past two decades, researchers spent significant amount of time in developing new microfluidic components for fluid transport, fluid metering, fluid mixing, valving, or concentration and separation of molecules [6]. Today, there are several types of microfluidic components that have already been created [6].

Microfluidic cartridges are commonly used in the biomedical field, in cell biology, and protein crystallization. [7]. Even more specifically in the biomedical field, it is used for drug delivery, cellular analysis and tissue engineering, and in diagnostic sensing applications [8]. Devices from these specific areas such as microneedles for drug delivery, single cell trapping and automated micro-robotic injection, and immunoassays for protein analysis on nanobioarray cartridges are typically used [8]. Interestingly microfluidic cartridges are being used specifically for blood analysis [9]. One example is a microfluidic cartridge that separates and collects white blood cells and red blood cells for microelectromechanical systems technologies, like micrototal analysis systems (μTAS) [9]. Other devices such as POC and microelectromechanical systems (MEMs) diagnostics can be used to determine the properties of blood [10].

Fluid movement and its integrity are dependent on the system and channel design. Currently, the microfluidic cartridge gold standard for blood analysis are composed of capillary driven microfluidics and thin-film electrodes for detection used by i-STAT [11]. Furthermore, the fluid movement done by capillary action through an open system where the liquid's diffusion is due to the adhesion of the molecules to the wall material from great hydrophilicity [12]. This can be represented through Washburn's equation as shown in Equation 1.

$$V = AS\sqrt{t} \tag{1}$$

Where A is the cross sectional area, S is the sorptivity of the medium, and t is time. [13]

One interesting mechanism that can aid in the design of microfluidic cartridges is the use of on-chip vacuum [14]. Vacuums allow for the fluid movement through the microfluidic cartridge by extracting the air in order to avoid the backflow of the fluid caused by air bubbles [14]. The amount of suction affects the rate at which the flow moves and potentially the amount taken. Hence, microvalves are needed to cease the amount drawn into the vacuum. Although it is efficient in extracting air, it can also disrupt the vacuum itself if the fluid has contacted the electrical components [14].

Pumps are another form of increasing fluid movement. The available types of microfluidic cartridge pumps are: syringe pumps, self-priming micro pumps, peristaltic pumps, and piston pump systems [15]. Interestingly, peristaltic pumps have been known to more commonly be used in

microfluidic cartridges due to the more fitted dimensions, easily being able to set-up, infinite amount of liquid that can be dispensed, and re-injection ability of the sample [16]. There are two types of peristaltic pumps which are hose pumps and tube pumps [17]. A peristaltic pump provides variable flow and a life performance [17]. The flow rate of the peristaltic pumps is known to be dependent on tube inner diameter, pump head outer diameter, and pump head RPM [17].

Microfluidic cartridge channel designs can also influence the rate and integrity of the blood cells. Several designs have been created and more specifically to continue the laminar flow in a cartridge the design used are done so that channels are fit to the limited size of the cartridge [18]. Furthermore, the channels are intentionally curved instead of having a 90° angle turning pathway [18] in order to prevent hard corners that may injure or lyse cells.

The fluid movement through a micro system like this can be characterized as laminar, simulating the body's fluid flow where there is no mixing within the layers of fluid [19]. This can represented using Reynold's equation:

$$\mathrm{Re} = \frac{\mathrm{D}v\rho}{\mu} \qquad (2)$$

Where ρ is the fluid density, D the tub diameter, v, the average fluid velocity, and μ the fluid dynamic viscosity. This is significant in terms of describing whether the fluid is in a laminar state or not; Reynold's number for laminar flow is 2300 [13].

The velocity of the fluid is also a significant factor in terms of rate of movement in a microfluidic cartridge that can be calculated taking into account of the wall shear stress as shown in Equation 3 [13].

Shear Stress at wall, r = R

$$\tau_{wall} = -\frac{2\mu\, v_{max}}{R} \qquad (3)$$

To represent the whole or part of a microfluidic system, then it can be modeled through Bernoulli's equation that assumes blood is continuous, incompressible, steady state, and isothermal. This can determine the velocity, pressure and height change that can occur as shown in Equation 4, where *P* is pressure, ρ is fluid density, *g* is the gravitation constant, *y* is fluid height, and *v* is average fluid velocity[13].

$$y_1 + \frac{v_1^2}{2g} + \frac{P_1}{\rho g} = y_2 + \frac{v_2^2}{2g} + \frac{P_2}{\rho g} \qquad (4)$$

In summary, these equations play an integral part in understanding fluid behavior at the microfluidic level – they provide the basis of upon which we can predict conditions that will impair our cartridge performance or even compromise our sample. It is with this in mind that we sought to design a chip that will promote laminar flow while reducing shear stress at the wall of the microchannel. With this in mind, we went on to design our cartridge to the specifications to be

mentioned later in this report. Additionally, the Computational Fluid Dynamic (CFD) add on in SOLIDWORKS use these equations to provide the fluid modeling that allowed us to predict areas of potential large shear stress that would cause damage to the sample.

2.2 Blood

Blood is the liquid connective tissue that transports oxygen, carbon dioxide, hormones, and nutrients throughout the body. The adult human body contains approximately 4-6 liters of blood, making up to 7% of a person's weight [20]. It is the major transport medium for oxygen, carbon dioxide, nutrients, and waste in the blood. Mechanically pumped by the heart, deoxygenated blood is deposited into the left atrium where it is pulsated into the left ventricles before returning to circulation via the pulmonary artery as oxygenated blood. The diagnostic value of blood is limitless due its integral purpose in the body as its major transport medium.

2.2.1 Constituents

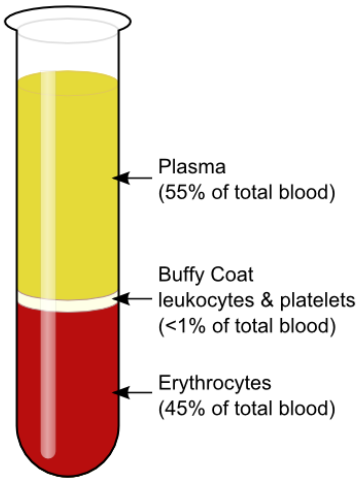

Figure 1: Constituents of Blood

Blood itself is a liquid matrix that consists of three main components which can be optically identified once centrifuged: plasma, the buffy coat, and red blood cells. Red Blood Cells (RBCs) are of particular interest to us as they determine the O_2 levels of blood, a parameter we hope to measure with our system. Red blood cells (RBC) or erythrocytes are produced in the bone marrow. They contain hemoglobin that allows for them to transport oxygen and carbon dioxide throughout the body, but lack a nucleus when reaching maturity. Measuring at 7-10 µm in diameter, RBCs have a lifespan of only about 120 days only to be removed by the liver and spleen. Their geometric design has allowed them to maximize surface area for gas transport while allowing them to reach the smallest of capillaries.

2.2.2 Panel Values

In the context of this project, blood yields blood gas and ion values that provide invaluable diagnostic information to healthcare providers. *Table 1* lists the acceptable blood panel values for blood gas, pH, and electrolyte values as stated by the U.S. National Library of Medicine provided by the National Institutes of Health [21].

Table 1: Blood Panel Ranges for a Healthy Human Adult

Blood Panel Parameters	Ranges
Partial Pressure of Oxygen (pO_2)	75-100 mmHg
Partial Pressure of Carbon Dioxide (pCO_2)	38-42 mmHg
Calcium (Ca)	8.5-10.2 mg/dL
Chloride (Cl)	96-106 mEq/L
Potassium (K)	3.7-5.2 mEq/L
Sodium (Na)	135-145 mEq/L
pH (arterial)	7.38-7.42

It should be noted that arterial blood has the truest value; however, studies have shown that it can be replaced with venous blood samples, which are much safer and easier to collect [22]. Deviations beyond these ranges may reflect a medical condition that should be addressed, such as kidney or liver failure. Thus, it is strongly important that diagnostic blood analyzers provide accurate values within a very small margin of error.

2.2.3 Mechanical Properties

Blood is a non-Newtonian fluid with a variety of mechanical properties stemming from its complex composition and its dynamic movement throughout the body in order to obtain a holistic perspective of its importance.

Laminar Flow

Blood flow in the body is generally laminar, characterized by blood moving in parallel layers (lamina) along the length of straight blood vessels under steady flow. Thus, blood moves with the lowest velocity (V = 0) at the blood vessel wall but moves with the highest velocity at the center of the vessel. *Figure 2* below illustrates the parabolic flow profile for laminar flow.

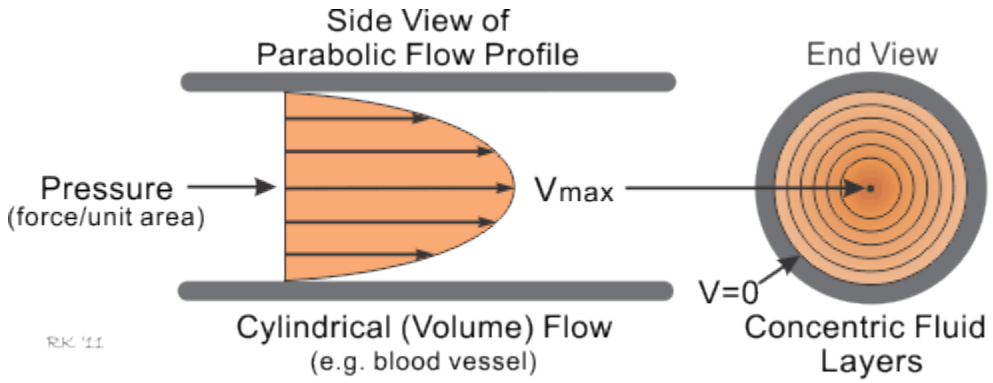

Figure 2: Profile and End View of Laminar Fluid Flow

Turbulent flow exists in the larger blood vessels like the large arteries at branch points such as the ascending aorta. Instead of steady parallel layers for flow, the layers become chaotic and may induce hemolysis. Turbulent flow increases the loss of energy via friction and generates heat, therefore more pressure is required to maintain the same flow rate. The transition from laminar to turbulent flow occurs at the critical Reynolds Number (Re) [23].

Reynolds Number

The Reynolds number of blood, which dictates the transition from laminar to turbulent flow and vice versa, varies with blood vessel type, as described in Table 2.

Table 2: Different Reynolds Numbers for Various Blood Vessels[24]

Vessel	Flow Rate (cm/sec)	Diameter (cm)	Mean Wall Shear Rate	Reynolds Number
Aorta	48	2.5	155	3400
Artery	45	0.4	900	500
Arteriole	5	0.005	8000	0.7
Capillary	0.1	0.0008	1000	0.002
Venule	0.2	0.00	800	0.01
Vein	10	0.5	160	140
Vena Cava	38	3.0	100	3300

As noted in the Microfluidics section of this review, the Reynold's number increases with vessel diameter as clearly reflected in *Table 2*. The Vena Cava, for instance, has the lowest shear rate but one of the highest Reynolds numbers. Turbulent flow is possible even at physiological flow velocity due to nonlinear blood vessels. A well-known anatomical instance of this is the use of a stethoscope to hear turbulence caused by physiological murmurs [25].

2.2.4 Density, Viscosity, and Clotting

Blood itself has many mechanical properties worth noting outside of laminar flow. The viscosity of blood depends not only on the type of blood vessel, but also on the presences of clotting factors. The relative density of blood (in comparison to water) is measured to be 1.06 $gm.cm^3$ and the surface tension is measured at 50 dynes/cm at 20°C [20]. The density of blood is proportional to hematocrit and total protein concentration, and both values closely resemble those of water.

Plasma itself, a Newtonian fluid, has a relative viscosity of approximately 1.8. The addition of other formed elements increases the viscosity. However, blood as a whole suspension is a non-Newtonian fluid and thus the viscosity is dependent on flow. Low flow states have been known to increase viscosity in order to promote molecular interactions between red blood cells and plasma proteins and between red blood cells themselves. This is due to the fact that RBCs adhere to each other to form chains in a process known as Rouleau formation [26]. Therefore, the presence of RBCs, which is characterized by a blood sample's hematocrit (%), drastically effects the viscosity. Normal hematocrit levels in the range of 40-45% corresponds to a viscosity of 40/100 millipoise or a relative viscosity of 4-5 [26, 27]. However, with increase in hematocrit, relative viscosity increases exponentially. Compounded with this process is the platelet aggregation and plasma protein interactions such as fibrin maturation that can also increase viscosity over time. Finally, blood also responds with a 2% increase in viscosity to every 1°C temperature decrease [26]. The culmination of these factors is described in the microcirculation by the Fahraeus-Lindqvist effect. In microcirculation (defined as circulation in vessels less than 200 microns in diameter) greatly reduces viscosity due to lower hematocrit enough to offset the increase in viscosity caused by lowered velocity. The next effect is that there is lower viscosity than predicted by typical viscometer measurements.

2.3 Biomaterials

The materials used in a device determine its physical and mechanical properties. When fabricating a biomedical device biocompatibility (the lack of a negative reaction to or with biological material) is a

required. In the case of microfluidic devices, an adverse reaction with any fluid could clog the micro-channels and render the entire device useless.

2.3.1 Base Materials

Microfluidic devices have been fabricated from many different materials. Advances in polymer manipulation and customization in the early 2000's have allowed for two biocompatible materials to come to the forefront of microfluidic design: Polymethylmethacrylate (PMMA) and Polydimethylsiloxane (PDMS) [28].

PMMA

Discovered in the 1930's, PMMA was initially used for aircraft windows during World War II and is most well know today in the medical field for its use as bone cement [29] [30]. PMMA is used as the base material in microfluidic devices and is limited by its reactivity to extreme pH and dissolution in ketones and esters [31]. PMMA has good blood compatibility and does not absorb proteins, limiting the blood's ability to clot.

PDMS

Recognized in 2002 as a significant material in microfluidic device synthesis, PDMS is the current base material due to its low cost, good material properties and multitude of fabrication techniques as shown in *Figure 3* [32].

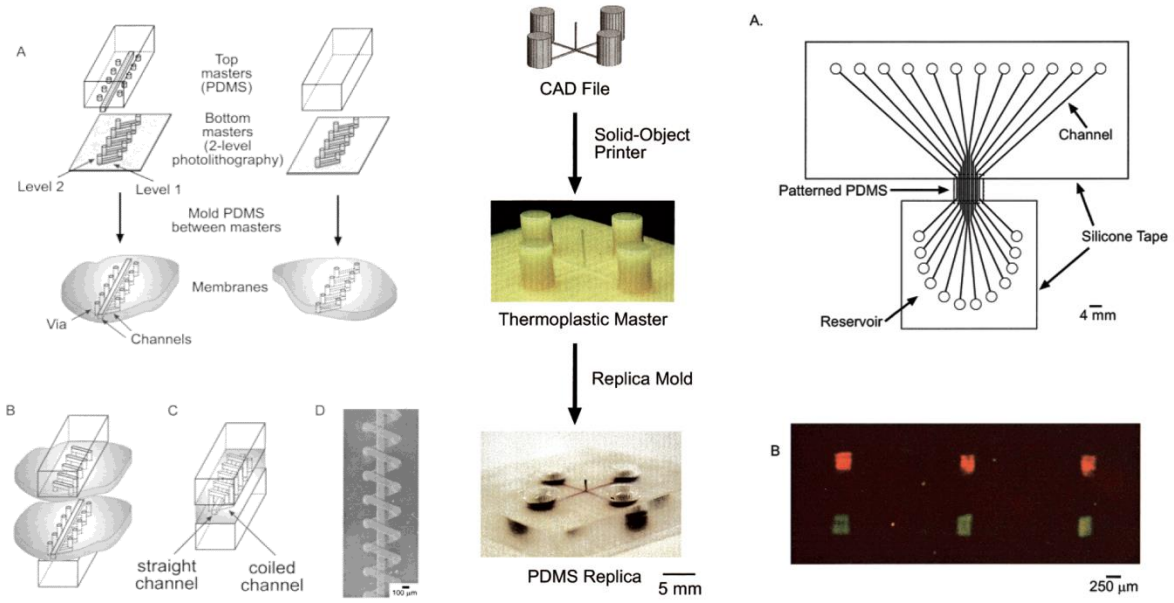

Figure 3: Fabrication Techniques of PDMS (left to right): 3D fabrication with stacking multiple layers, with 3D printed thermoplastic master, with etching of inlets and outlets[32]

Flexibility of fabrication is extremely important especially when it comes to microfluidic devices and channel design. These channels have been used for differentiation of tumor cells using a herringbone pattern to the separation of plasma from whole blood via axial migration [33] [34].This research led to many advances in diagnostics including a recent publication demonstrating the impact of low-powered, cartridge-based point of care immunoassays, nucleic acid analysis, and detection on quick and reliable testing without the need of expensive lab equipment [35]. These recent collective insights have led to more unconventional materials to come to the forefront of microfluidic design.

2.3.2 Recent Innovations

The need for cheap, accurate, and disposable chemical analysis tools combined with the recent advances in printing technology has brought about the latest microfluidic sensing base material [36]. While this application does require a specific type of paper, one made of pure cellulose with no additives such as chromatography or filter paper, the printing is extremely versatile and can use a myriad of printing techniques to create the desired sensor or assay. *Figure 4* demonstrates the possibility for multiple detection assays on one device as well as the process for printing and interpreting the results.

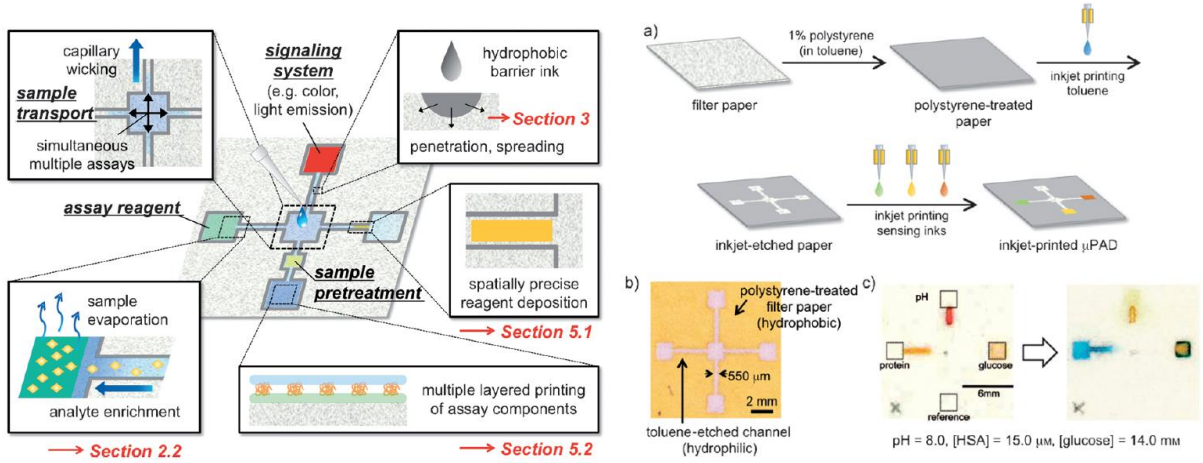

Figure 4: Schematic representation of paper based analytic device,(a) Methods to create microfluidic channels on paper substrates, (b) creation of hydrophobic barriers, (c) what each block detects and how to read the results[36]

Not only does this form of testing have the advantage of multi-functionality, it also allows for customization by the manufacturer based on the range of analytes to be detected. Proteins and other large biomolecules are typically hard to detect; however, recent advances in printing in 3D has allowed for a multidimensional entrapment layer tailored for these molecules to be designed and fabricated. A more detailed explanation of the process of enzyme entrapment for the detection of proteins and other large biomolecules is described in *Figure 5*.

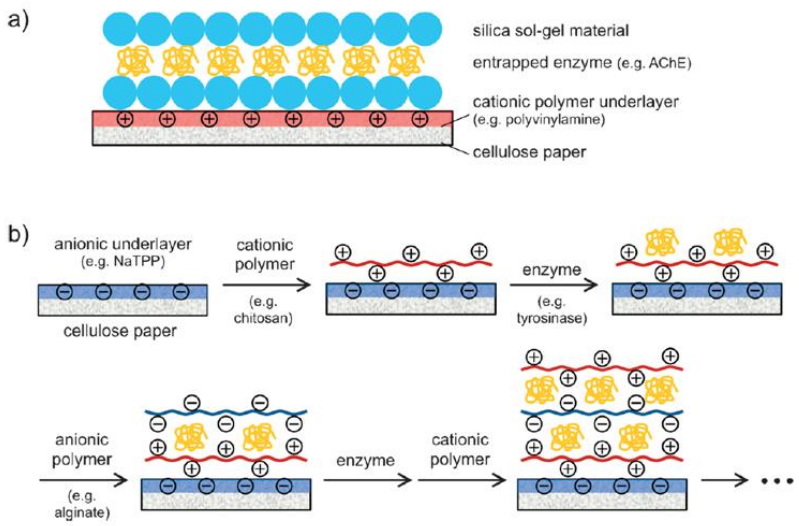

Figure 5: Strategies for enzyme entrapment and stabilization using ink-jet deposited layered structures, a) silica sol-gel materials, b) a pair of positively/ negatively charged polymer materials [36]

The mentioned processes have opened the door for this technology to be used in many different fields. One study has used these devices to detect antibiotics and heavy metals for the food processing industry [37]. These paper devices are allowing for the cost of detection to decrease dramatically and are pushing the limits of performance of conventional printing techniques. The final advancement to be covered in this section can be used either disparately or in tandem with existing technology to advance the field of microfluidics to a new height.

Nanomaterials

Nano-scaled materials and particles have increasingly become popular for uses in multiple fields of research due to their high surface to volume ratio as well as their desirable customizable properties. They can be used for a range of applications including: sensors and transducers, antibacterial agents, antimicrobial textiles and food packaging to name a few. On the biomedical side they are being explored for their uses in biosensors as: glucose sensors (bimetallic core shell of PEEK and Pd, Pt, Co, Ni composites), amperometric sensing of urea (PANi nanocomposite stabilized with PVA), and to sense dopamine (PEDOTA/Au composite) [27]. In the context of microfluidic devices, magnetic nanoparticles allow for flexibility in design due to their ability to be used for manipulation of a fluid (i.e. stirring, positioning) with ease over a magnetic inductor. Internal sensing has also been enhanced with the use of gold and silver-silicon particles as well as carbon nanotubes [38]. Another silver nanocomposite has been recently researched for its antibacterial properties as well as its ability to increase blood compatibility of a material [39]. Nanomaterials are not only limited to nanoparticles, recent advances in microneedle fabrication techniques have allowed for their integration as a means of collection of sample for microfluidic analysis. This combination is completely redefining the limits of sensing materials and technology.

2.4 Sensors

Current IL technology uses miniaturized sensors to measure blood analytes such as pH, pCO_2, pO_2, Na^+ sodium, K^+ potassium, Ca^{++} calcium, hematocrit, glucose, and lactate. The sensors are described as "maintenance free, high reliability and flexibility, and cost efficient,"[40]. The current

sensors in IL's newest bench top tester, the GEM4000, requires 150 µL of undiluted whole blood and 50 to 55 seconds for sensor analysis. Additionally, IL's current line of blood gas analyzers use electrochemical sensors that are hydrated and calibrated prior to use[41].

Electrochemical sensors respond to the permeation of the analyte and the difference between the conducting and non-conducting material [42, 43]. These differences can be based upon potentiometric, amperometric, or conductivity sensing [43]. For medal oxide sensors, the electrical resistance changes according to the presence and concentration of the analyte. The metal needs to be heated for chemical detection therefore there is a thermal block that maintains a 37°C temperature and provides a thermal interface.

Within the blood gas analyzer cards used in IL's current bench blood analyzers, there is an array of electrochemical sensors in a gas tight chamber while using principle ion-selective electrodes and silver ions Ag/Ag+ as reference electrodes. *Figure 6*: Ion-Selective Electrode shows a layout of the ion-selective electrode used with the sensor chamber. The silver reference electrodes proves to be stable, leading to more reliable sensor measurements. As an example the GEM Premier 3000 has an open liquid junction between the Ag/Ag+ electrode and the sensor chamber. The Ag/Ag+ flows through small fluid path to come in contact with unknown the sample solution [42].

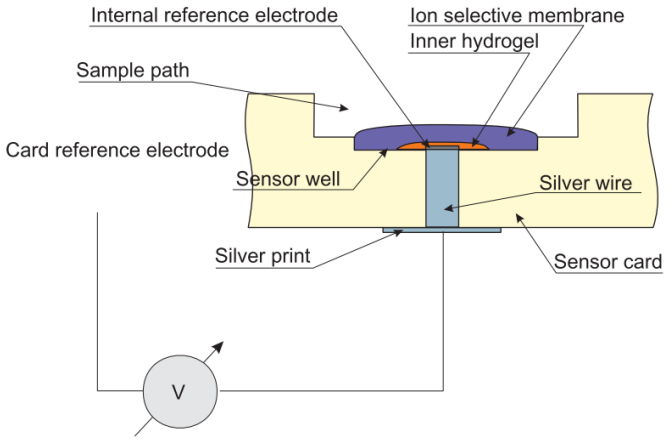

Figure 6: Ion-Selective Electrode

The pO_2 sensor is an amperometric electrode [40], analyzing the current at the junction which is linear to the concentration of the analyte [43], in this case oxygen. *Figure 7* shows a cutaway image of the oxygen sensor. Redox reactions occur at the sensor interface, causing the transfer of electrons and a current flow [43]. The sensor uses negative potential platinum electrodes with the silver reference electrode. The electric current changes based on the detection of ions in the solution. For pO_2, the current between the platinum electrode and ground is equal to the oxygen partial pressure. It is also important to note that the gas permeable membrane protects the platinum from blood contamination.

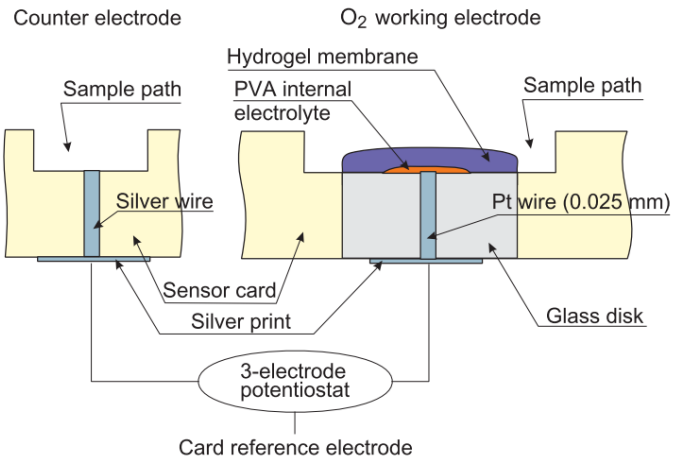

Figure 7: Oxygen Sensor

The pCO_2 sensor uses a pH selective polymer as a gas permeable membrane on the outside. The pCO_2 and the internal bicarbonate buffer come to equilibrium, cause a change in pH value and generating a potential against the pH sensor. That reading correlates with the log of the pCO_2 in the blood sample [40]. Therefore, this design acts as both the pCO_2 sensors in the array. *Figure 8*: Carbon Dioxide and pH sensors shows both sensors with the internal bicarbonate buffer, pathways, reference electrodes, and pH selective membrane.

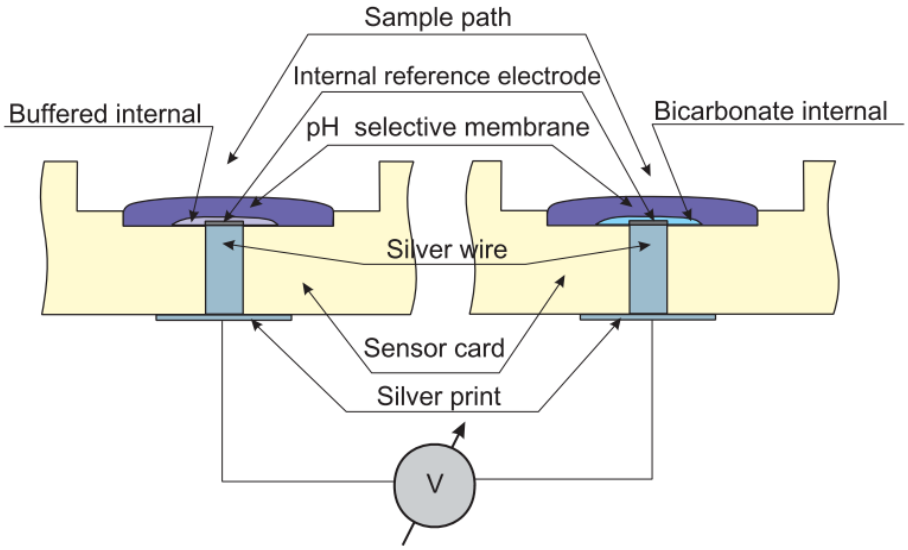

Figure 8: Carbon Dioxide and pH sensors

Hematocrit sensors are conductometric sensors, measuring the electrolyte conductivity based on the environment it is exposed to and the number of charge carriers (ions/electrodes) in the electrolyte [43]. As the concentration of cells in the blood increase, the resistance of the cell membrane also increases. The hematocrit sensor uses two electrodes and alternating current, measuring the resistance of the electrolytes using Ohm's law as the hematocrit affects the plasma resistance value [40].

Finally, metabolite (glucose and lactate) sensors are also amperometric sensors measuring electric current. With these sensors the platinum electrode has a positive potential rather than a

negative potential. There are three layers for this sensor. The inner layer is the screen interface, followed by the middle later of enzymes for an oxidation reaction. The outer later controls the metabolite diffusion from the blood sample into the enzyme later. A glucose or lactate oxidase enzyme reaction results in H_2O_2 and a current. That current between the electrodes (platinum and ground) is proportional to the concentration of glucose or lactate, thus determining those levels.

These sensors have gone through quality assurance, tested to assure reliability. There is an analytical performance evaluation of the sensors (used in the GEM Premier 4000) for aqueous control precision, serum accuracy, blood precision, and whole blood accuracy. pH, pCO_2, pO_2, Na^+ sodium, K^+ potassium, Ca^{++} calcium, hematocrit, glucose, and lactate, are useful for blood gas analysis and critical care biomedical instrumentation.

2.5 Competing Products

POCT has allowed physicians to immediately perform clinical testing at the patient bedside rather than sending samples to a separate laboratory for processing. Not only has this trend reflected the great advancement of technology, but also the advancement in diagnostic care. POCT offers quick and reliable results to physicians and nurses in order to allow for immediate clinical decisions. Many clinical tests were already bought to the bedside and even to the home (e.g. pulse oximeters, glucose testing, and urine testing).

2.5.1 Bench Top Blood Gas Analyzers

One continually emerging market is blood gas analyzers. There have been many bench top models produced by many companies such as Abbott Laboratories, Siemens, and Instrumentation Laboratory. They aspirate 100 μL or less of sample blood from a syringe or capillary tube, and perform analytic testing and yield blood gas and electrolyte values such as pCO_2, pO_2, pH, Na^+, K^+, and Cl^-. Many others are capable of measuring additional metabolites such as glucose, lactate as well as CO oximetry. Other blood values may be derived from the measured analytics, such as TCO_2, Ca^{++}, and CaO_2 as the GEM Premier 4000 produce by Instrumentation Laboratory does. These tests can be performed in 3 minutes with most analyzers or even as fast as the only 1 minute as with the Siemens RAPIDLab 1200 system as shown in *Figure 9* [44]. The major advantage of these analyzers is that they are capable of providing reliable results from small samples in a very short amount of time, allowing them to be used in intensive care units (ICU), neonatal intensive care units (NICU), and emergency departments (ED).

Figure 9: Siemens RAPIDLab 1200 Systems (Not available in the US) [44]

These analyzers typically consist of two main components: an electronic recording module and a disposable cassette. The recording module contains the micro-circuitry, reagents, pumps, and other non-disposable components. These modules read, interpreted, and recorded the blood gas analysis while also performing quality control on the machine itself to ensure accuracy. Many are even capable of connecting to the medical facility's patient record system to input the readings. However, the disposable cassette contained the testing reagents, sensors, and calibrators for quality control. They may also contain disposable pouches for storing waste produces of the analysis. These cassettes were removed after approximately 30 days or after a certain number of tests and disposed for biohazard removal. The device overall not only limited user exposure to biohazard, but performed all of the necessary blood gas testing within a medical department.

2.5.2 Handheld Blood Gas Analyzers

Despite the advantages of tabletop gas analyzers, they pose many difficulties in faster paced environments such as field hospitals, emergency departments, and even the home. They are typically meant to be stationery units within a hospital wing. In current times, several companies have produced affordable mobile units capable of performing the same tests as their desktop counter parts. Abbot Laboratories and Alere$_{TM}$ have both developed similar products that are capable of performing tests on 100 µL of blood or less within one minute [2] [1].

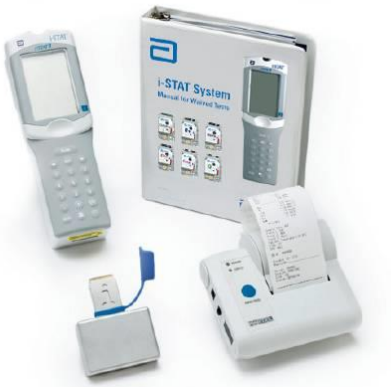

Figure 11: The Abbott i-STAT blood gas system with cartridges and printer

Figure 10: Alere$_{TM}$'s epoc® blood gas analysis system with cartridges

These systems too are composed of two main components: an electronic module and a disposable cartridge. The electronic module again, can record, calibrate, and read the sensors and transmit the information via WIFI, Bluetooth, or RFID to the patient record system at the hospital. They may also contain the mechanical components to incite fluid movement such as pumps and vacuums. The cartridge on the other hand is where the blood sample may be place either through a finger prick with a lancet or with a syringe. The cartridge is then inserted into the electronic module which then analyzes the sample. These analyzers offers point of care testing to the patient bedside and performs tests in the matter of seconds.

Despite the many advantages of these systems, they still pose several setbacks. For instance, the i-STAT cartridges not only must be refrigerated (5-8°C) until use, it has a very short shelf life. Additionally, the cost per test can be as high as approximately $37.00 for a single By-type natriuretic peptide (BNP) or two site enzyme-linked immunosorbent assay (ELISA) reading, to about $4.98 per test for a single glucose reading[45]. The EC8+ cartridge provides the blood panel closest to that desired by IL: Na, K, Cl, glucose, urea nitrogen, hemocrit, hemoglobin, pH, PCO_2, and PO_2 at the cost of $17.53 per test[46]. It is our hope that not only would we lower the cost per test of our device, but also allow integration of IL's current sensor technology, allowing the company to become a contender in the POC market.

3. Project Strategy

3.1 Clarification of Original Problem Statement

The team was given the following problem statement in a project given by the project sponsor, Instrumentation Laboratories (IL):

- Design and develop a micro-fluidic disposable cartridge to be used in a hand-held blood analyzer.
  - Increase portability
  - Reduce sample volume
  - Simplify fluidic control
- Can be used with current sensor IL technology and formulation.
- Opportunities for future product expansion

3.2 Objectives and Constraints

Upon the first meeting with the clients at Instrumentation Laboratories, the team was asked to design a handheld device that leverages a disposable microfluidic cartridge utilizing existing IL sensor technology to complete analysis on whole blood. This objective was followed by the following list of design more specific design requirements:

1. The device shall complete analysis in less than 3 minutes
2. The device shall maintain blood sample integrity (eliminate contamination air and reagent, reduce hemolysis)
3. The device shall position the patient blood sample repeatedly over blood sensors
4. The device shall complete analysis on whole blood
5. The device shall complete analysis with a blood sample no larger than 100 μL
6. The device shall fit in the palm of a user's hand
7. The device shall measure Chloride, Potassium, Calcium, pO_2, Sodium, pH, pCO_2, using existing IL sensor technology
8. The device conveys current test status to user
9. The device shall be able to aspirate blood from a syringe
10. The device shall have a disposable single use sample component
11. The device shall limit operator exposure to biohazard
12. The disposable component of the device shall cost less than 1/10 of typical analyte/test reimbursement
13. The disposable component shall interface with the device electronics to support sensor function
14. The device shall be RoHs compliant
15. The device shall comply with IEC 60601
16. The device shall perform analysis at body temperature (37.4 +/- 0.3°C)
17. The device leverages open source electronic platforms such as Arduino, Raspberry Pi, Beaglebone Micro controllers
18. The device shall operate in any handheld orientation

With this list of requirements, the team sought to understand the overall description they envisioned of this device. These criteria were broken down into three main categories as displayed in *Figure 10: i)* a disposable component, ii) the analysis of sample, and iii) interaction with operator.

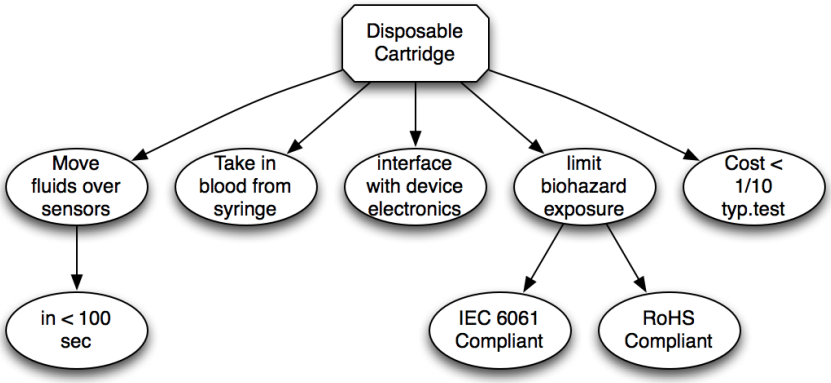

Figure 12: Category 1 - Disposable Cartridge Break Down

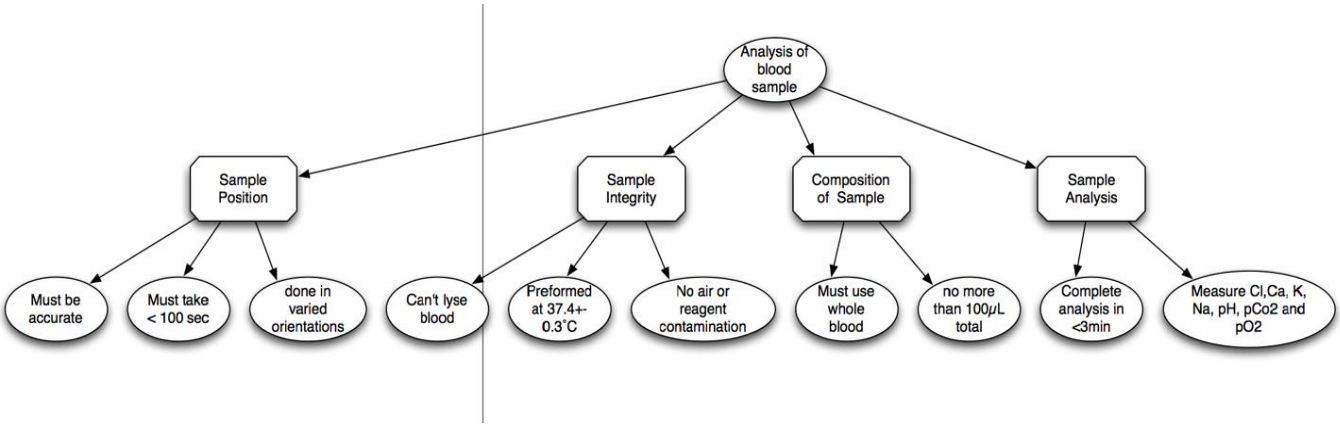

Figure 13: Category 2 - Analysis of Blood Sample Break Down

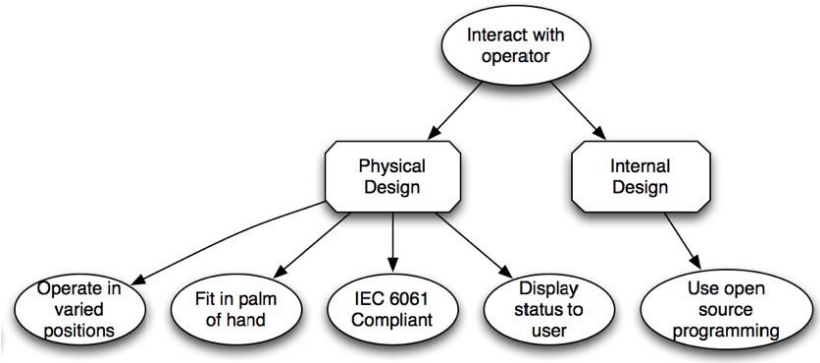

Figure 14: Category 3 - Interactions with Operator Break Down

3.3 Revised Problem Statement

The second step was to prioritize the requirements based on the importance to the functionality of the device and to create sub requirements for each "top priority" design criteria to ensure the functionality and constraints were met. The top priorities determined by the team are listed with their sub requirements below.

Break Down of Top Priority Requirements

The device shall position the patient blood sample repeatedly over blood sensors.

The device should consistently have a constant laminar flow, pressure, and constant volume with a Reynold number is 2300 or less [1]

Blood pressure should simulate physiological conditions, therefore ranging from 80 – 120 mmHg [1]

The device shall complete analysis with a blood sample no larger than 100 µL.

Testing must be done in order to determine total amount of sampling blood used/lost:

In sensors (~1.8 to 10 µL per sensor) [3,4]

Through pathway of channels (Pressure, velocity, blood viscosity/thrombin, diameter of channels, and material coating can vary the amount of deposit) [5,6]

Due to denaturing/apoptosis (depending on inner layer of channels, pressure, and velocity) [10,11]

The device shall fit into the palm of the user's hand

The device shall be no greater than 160 mm wide [7]

The device shall be no heavier than 0.4 kg [8] per OSH standards (but compared to cellular devices should be no heavier than 140g)

The device shall not force the rotation of the wrist more than 120° from pronated neutral position [7]

The device shall retain a increased friction material covering the back surface [7]

The device shall have a disposable single use sample component

The disposable component shall limit user biohazard exposure

The disposable component shall move whole blood reliably over sensors in under 55 seconds

The disposable component shall adhere to all other major requirements

The device shall limit user exposure to biohazards

The disposable component shall be completely sealed before and after use

The connection between the disposable component and the device shall only be electrical and will contain no biological fluids

The device shall complete analysis in less than 3 minutes

Must be able to measure all of the following using sensors from Instrumentation Labs: Chloride, Potassium, Calcium, pO_2, Sodium, pH, pCO_2

The sensors will run simultaneously for 50-55 seconds after the blood is on the sensors

Use a reliable, fast pump to move the blood through component to the sensors without interfering with the sample's integrity

The disposable component of the device shall cost less than 1/10 of typical analyte/test reimbursement
The material of the component should be inexpensive enough to dispose of
The material should be easily accessible and made to the component's shape at a low cost
The disposable component will be less than $15.00 [9]

The device shall maintain blood sample integrity
User must introduce the blood sample to the device within 3 minutes of extraction
Samples must be processed within device under 300 seconds in order to ensure minimal clotting
Suggested microchannel diameter for most applications was between 70-100 μm minimum for general whole blood flow
A suggested shear rate of 220 s^{-1} or lower should be introduced
Suggested flow rate of 0.250 mL/min in order to prevent hemolysis
One analysis procedure will not change the composition of the sample when it moves on to the next analysis procedure.
The design must avoid 90 degree angles or bifurcates with different flow rates as this may cause cells to separate from plasma
Sample will not be combined with other reagents while in processing cartridge

The device shall complete analysis on whole blood.
Blood will be fresh from patient with no reagents added
Sample must be extracted from center of syringe in order to prevent elevated pO_2 and decreased pCO_2 levels

3.4 Project Approach

In order to execute this project, the team established sub-tasks under each of the three major category of needs as well as market needs:

1. Design a disposable microfluidic cartridge to transport blood onto sensors
    a. Test the amount of fluid loss across the known length of channel to sensors
    b. Choose a corresponding pressure, flow rate, and channel diameter that will ensure maintenance of blood sample integrity
    c. Design channels that will allow for repeated accuracy of blood placement over the blood gas analysis sensors
    d. Design and implement additional sensors that will monitor flow of blood through channels
2. Analysis of Blood Sample
    a. Ensure that blood gas and electrolyte sensors are compatible with placement in disposable microfluidic cartridge
    b. Validate functionality and accuracy of sensors within cartridge
    c. Optimize analysis procedure to perform all required steps within 300 seconds

    d. Run comparison tests with the current GEM 3000 model at IL
3. Interactions with user
    a. Ensure gas and electrolyte results are relayed to user
    b. Allow the device to notify user of device functionality such as testing status, fluid movement, and malfunctioning
    c. Establish safety mechanism in case of device malfunction to limit biohazard exposure to user

Therefore our team divided these goals into the following set subtasks:

1. Design a functional microfluidic cartridge capable to placing whole blood over sensors repeatedly without endangering the integrity of the sample taken from various specimen containers.
2. Optimize functionality of the analysis procedure in order to perform blood gas analysis within 300 seconds while having the device size to fit in the palm of a user's hand.
3. Relay results and status of analysis to the operator during use.

After much research, design, and communication with IL, our team decided to focus mainly on the positioning procedure and calibration of the microfluidic cartridge and its fluid movement mechanism rather than the sensor results and placement. This decision allowed us to fine tune the performance of the cartridge and ensure that this invaluable stepping stone would provide the foundation for the analysis portion of this project.

4. Design Process

After encompassing the user needs into a cohesive client statement, our team narrowed down the scope of our project to focus on the fluid movement mechanism and design of the disposable microfluidic cartridge component. With this in mind, we performed a detailed needs analysis and investigated different fluid movement mechanisms as well as different fluid modeling software. We re-evaluated our designs once more towards the conclusion of the conceptualization time period and chose our final design to pursue and fabricate: a microfluidic device driven by a syringe-stepper motor combination.

4.1 Needs Analysis

POC test systems allow patient diagnosis in different medical settings through easy and fast blood analysis. IL seeks to improve their POC test systems with the following objectives:

- Design a functional, disposable, inexpensive, and portable microfluidic cartridge.
- Performance is repeatable, consistent, and fast for whole blood gas analysis.
- Allow easy operator interaction through a handheld device.

Table 3: Pugh Matrix Ranking and Weightings

Micro-Fluidic Blood Analysis Cartridge User Needs	**Rank**	**Team Points Assigned**	**IL Points Assigned**
The device shall position the patient blood sample repeatably over blood sensors	1	5	5
The device shall maintain blood sample integrity (eliminate contamination air and reagent, reduce hemolysis)	2	5	5
The device shall complete analysis on whole blood	3	4	5
The device shall complete analysis with a blood sample no larger than 100 μL	4	4	5
The device shall fit in the palm of a user's hand	5	4	4
The device shall complete analysis in less than 3 minutes	6	4	4
The device shall have a disposable single use sample component	7	3	3
The device shall limit operator exposure to biohazard	8	3	3
The disposable component of the device shall cost less than 1/10 of typical analyte/test reimbursement	9	2	2
The device shall measure Chloride, Potassium, Calcium, pO2, Sodium, pH, pCO2, using existing IL sensor technology	10	4	2
The device shall be able to aspirate blood from a syringe	11	2	3
The device shall perform analysis at body temperature (37.4 +/- 0.3 C)	12	3	3
The device shall operate in any handheld orientation	13	2	2
The disposable component shall interface with the device electronics to support sensor function	14	3	2
The device shall comply with IEC 60601	15	3	3
The device conveys current test status to user	16	2	2
The device leverages open source electronic platforms such as Arduino, Raspberry Pi, Beaglebone Micro controllers	17	1	1
The device shall be RoHs compliant	18	3	2

With a thorough understanding of the functionality of the device and the design criteria by revising the problem statement in the previous chapter, the team went on to create a Pugh matrix weighting all of the design criteria on a scale of 1-5. A Pugh matrix according to SixSigma "is a tool used to facilitate a disciplined, team-based process for concept generation and selection. Several concepts are evaluated according to their strengths and weaknesses against a reference concept called the datum (base concept)." [45] In this matrix, each client need is weighed by importance on a scale of 1-5. Afterwards, each designed is graded again on a 1-5 scaled based on how well it accomplishes said requirement. These two values are then multiplied together in order to achieve a value that displays each design's relevancy in accomplishing the project's most important requirements. The team and IL separately completed a Pugh matrix regarding the importance of each project criteria as shown in *Table 3*. After several discussions between IL and the team, we came to the final client needs ranking. The project major focus was less on the analytical side but was chosen to focus more regarding the fluid

positioning system and accuracy of the device. These weighs eventually were used for comparison of the various preliminary designs, as elaborated in the following chapters.

Through analyzing the Pugh Matrix, the criteria can be broken down into three main functions of the POC test system: blood analysis, disposable cartridge, and operator interaction. These match our three set of subtasks as created in *3.4 Project Approach*. The first subtask was to consistently and accurately analyze a blood sample. Analysis of the blood can further be broken down into sample integrity and composition, positioning, and blood gas analysis. This task plays a critical role with our design. As a POC concept, the blood test would be used for diagnostics. Therefore, accurate blood positioning over the sensors and maintaining sample integrity while using whole blood are the highest ranked user needs in our Pugh Matrix. While considering our designs, we needed to keep in mind that the sample positioning must be repeatable, take no more than 100 seconds, and undergo varied orientations to prevent the least amount of interference with the sensor analysis. Our design would also need to maintain sample integrity by preventing lysing to perform analysis of whole blood, having no air or reagent contamination of the blood, and performing at body temperature of 37.4 +/- 0.3 ^{o}C. With these needs, our design would provide reliable blood gas analysis data, the main goal of the microfluidic device.

Another important function of the POC test system is having a small, low cost disposable cartridge. The chosen material and additional electrical components all determine its size, cost, and disposability. The device should also limit biohazard exposure and be IEC 6061 and RoHS compliant. These client requirements are needs but because they are flexible, they are not ranked as high as the blood analysis requirements. We considered these aspects throughout our design process, but prioritized blood sample analysis over these disposable component needs.

Interaction with the operator is also an important consideration when focusing on the exterior and interior design as well as ease of use. The analyzer must be able to operate in any orientation, quickly analyze the blood, and relay the results to the user while still fitting it within the user palm. These client requirements were ranked "wants" for the design rather than needs, since the other needs such as fluid positioning and analysis must first be accomplished before this device requirement could even be validated. Operator interaction conveying the test results and leveraging electronic platforms are considerations that can be dealt with towards the conclusion of this project and thus they were not considered precedence constraints for the design compared to blood analysis.

These needs were achieved through our final design that used a well-controlled system and was composed of biocompatible and inexpensive materials. Based on the rankings of the user needs and required qualities, we ordered the important needs and wants for conceptualizing design ideas and prototypes.

4.2 Important Industry Standards

There are two user needs that we must prioritize involving engineering standards which specify characteristics that our designs must need based on what the standards cover. Our device shall be compliant with RoHS (Restriction of Hazardous Substances also known as Directive 2002/95/EC) and IEC 60601 (International Electrotechnical Commission). Our device would utilize blood samples and therefore, besides being biocompatible with the sample, it must limit user exposure to biohazardous materials. Here is further evaluation of these standards and how they affect our design.

RoHS restricts a certain amount of hazardous materials found in electrical components. These materials include, but are not limited to lead, mercury, cadmium, and hexavalent chromium [47]. Our designs would not include any of the hazardous materials that are RoHS compliant. Moreover, the RoHS directive splits electrical products into certain categories such as large household appliance (category 1), lighting (category 5), and power tools (category 6). Medical devices and equipment are covered by Category 8 which are considered exempt from RoHS compliance [47], including our microfluidic blood gas analysis device.

The IEC 60601 technical standard assures the safety and effectiveness of medical electrical equipment [48], covering power supplies (both AC-DC supplies and DC-DC converters), procedure invasiveness, and operator/patient protection before being put to market. The power supply must be managed at proper currents and voltage to prevent injury to both the user and those by which the device is used upon. Safety measures and controls include a protective ground for energy and also isolation barriers [48]. *Figure 15* shows an overview of the testing procedure for IEC 60601 compliant devices. With this standard, we must be conscious of our power usage and how our device interacts with the operator and patient.

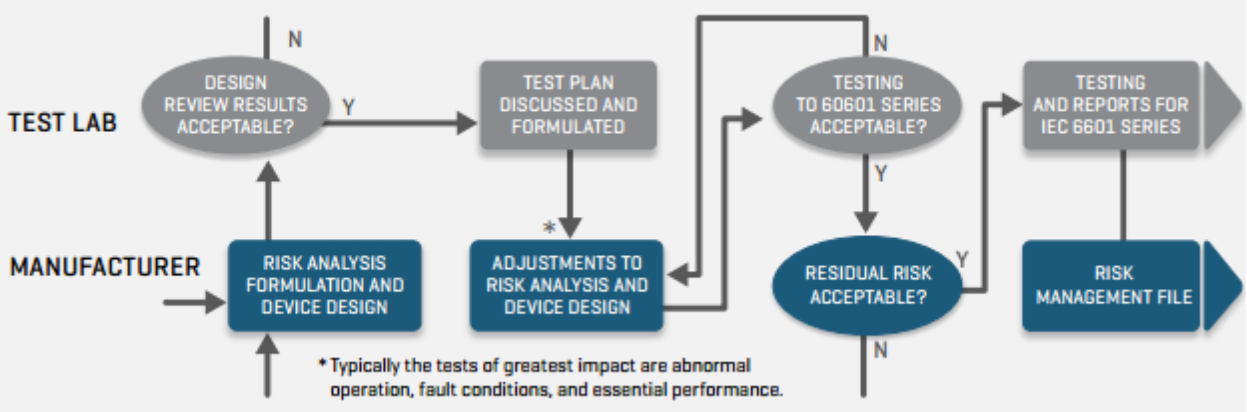

Figure 15: Overview of IEC Testing Procedure

Finally, since our device does utilize with blood, it must be biocompatible to prevent erroneous analysis results as well as limit user biohazard exposure. For these considerations, we looked at ISO (International Organization for Standardization) standards for biocompatibility (10993-1) and blood processing (ISO/TC 76). Our design must limit the exposure of blood to the operator and patient to prevent blood borne pathogen contact while still performing proper blood processing [49].

These standards impacted our device design to ensure that they are safe to use for the operator and patient by limiting biohazard exposure, maintaining proper electrical power usage, and not using hazardous materials while still giving reliable blood analysis data. These industry standards ensure that our device along with any others on the market will provide safe and reliable health care services

4.3 Preliminary Conceptual Designs

After analyzing and prioritizing the needs of our device, we sought to better understand the available options to accomplish them. In particular, we focused on different fluid movement mechanisms that would draw the blood into our device and position it accurately over IL's electrolytic sensors, while pulling the electrode reagents to a different part of the cartridge. We explored 5 major options: a differential pressure chamber, a peristaltic pump and capillary tube combination, a microneedle array patch with a pressure chamber, compressed air used with an air cylinder, and a

syringe pump. The following sections describe the mechanism of each design as well as our selection process for our final preliminary designs: the peristaltic pump and capillary tube combination as well as the syringe pump design.

4.3.1 Differential Pressure and Outlet

This design is based on the concept of pressure differential as the method for fluid movement. In both iterations of the design, the fluid containment chamber would be sealed in a vacuum to a specific pressure that would be released upon the breaking of the seal and cause the aspiration of blood. This seal would be created by either a rubber septum or a silicon micro-valve. The initial design, design 1.0, was created from research done on current phlebotomy (blood drawing) equipment and mimicked the current dimensions and set up of vacuum sealed drawing tubes. Design 2.0 was created as a refinement and focuses more on aspirating pre-drawn blood rather than patient interaction.

4.3.1.1 Iteration 1

The main focus of design 1.0, depicted in, was the ability to interface with the current phlebotomy equipment and therefore be able to directly interface with the patient. This straight draw eliminates many of the current problems of blood clotting due to extended wait time before analysis as well as the need for an external heating block to warm up the blood for analysis. The cylindrical obtrusion in design 1.0 is based off the dimensions of the currently used vacuum tubes for blood draw and is compatible with the butterfly needle draw system. In this protrusion, a rubber membrane is punctured and the negative pressure in the cartridge created by the vacuum draws in the blood over the sensors and removes the reagents that were sitting on the sensors, putting them in the waste reservoir. A green LED light would illuminate once the correct amount of blood is drawn in over the sensors, which would tell the user to remove the needle from the septum. Once the blood has been drawn in, the cartridge is then inserted into the handheld device for further analysis. The use of a septum seals the cartridge from the surrounding environment and allows the cartridge to fully contain all biohazardous material. Although design 1.0 may have many desirable qualities, is does have several drawbacks such as a single point of failure in the rubber septum, the need of a power source on the cartridge itself, an increase in risk of operator error in forgetting to insert the cartridge, and finally, the lack of the ability to directly aspirate blood from a container. With these design flaws in mind a second iteration of the cartridge was created.

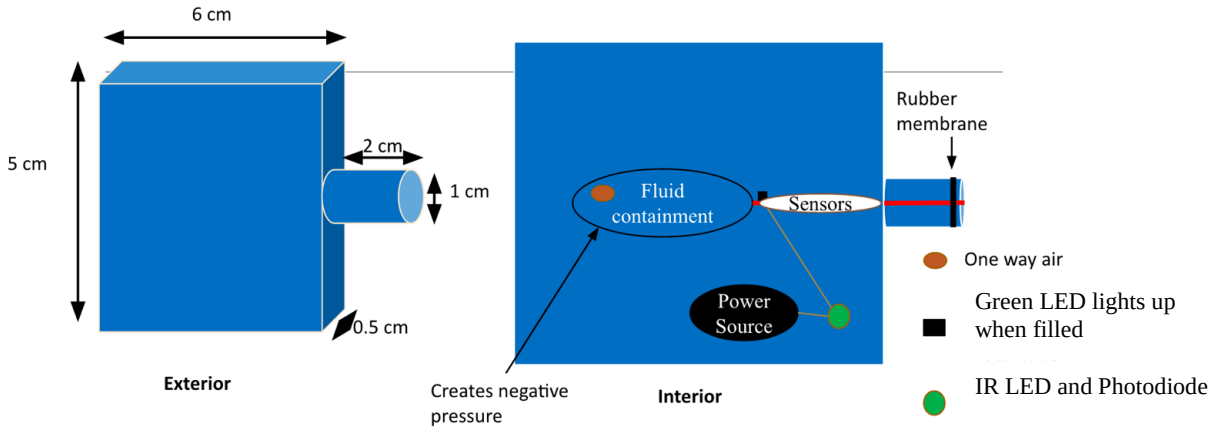

Figure 16: Differential Pressure Design 1.0

4.3.1.2 Iteration 2

The main focus of the second iteration was to enable the introduction of blood from many different containers through aspiration. Design 2.0, shown below in *Figure 17,* changed in both scale and shape allowing for smaller quantities of blood to be introduced and contained within the system.

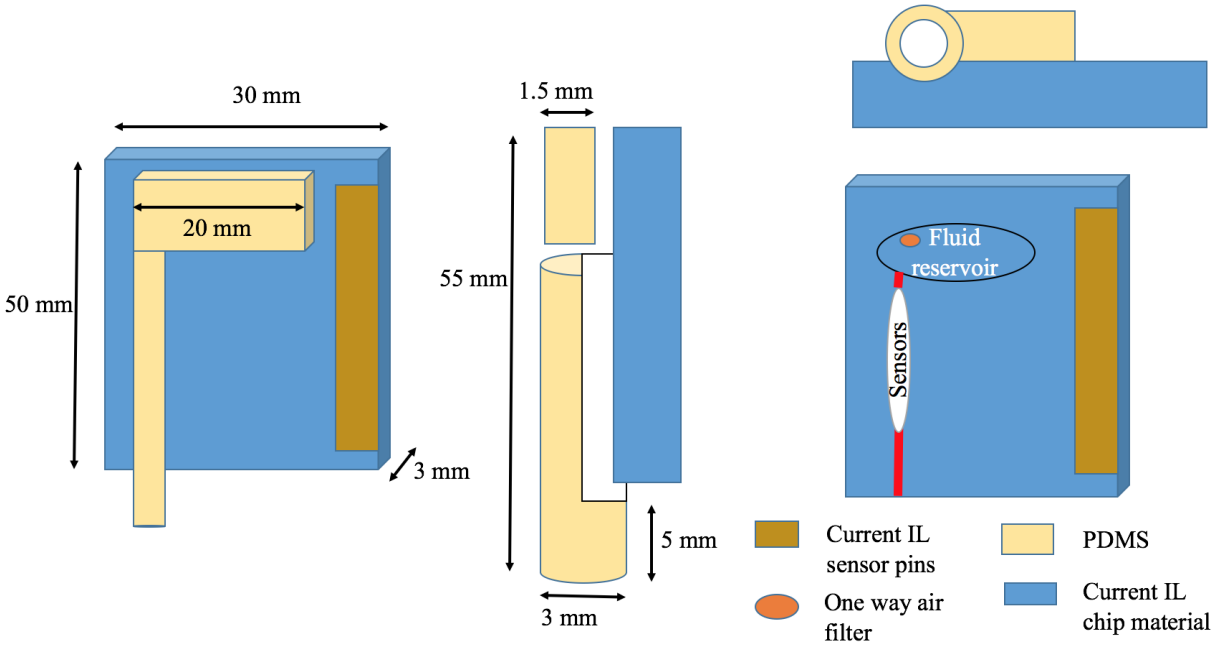

Figure 17: Differential Pressure Design 2.0

Fabricated in two different components, the base plate and the covering, this design would be compatible with current IL technology, not need an internal power source, and could be easily manufactured with just one additional step to the current manufacturing process. Fluid movement is again controlled by differential pressure created through vacuum sealing the base plate and the cover together, however in this design there is no rubber septum and instead there is a silicon micro-valve that can be decompressed by the handheld device to begin the aspiration process. The cylindrical protrusion in this design is used to be dipped into a container or drop of blood to allow for aspiration from multiple containers. The utilization of aspiration of small quantities of blood, such as the 100 µL produced by a finger prick [50], eliminates the necessity of using any phlebotomy equipment or the need of a specialist to draw the blood. Design 1.0 would work well for tests that require larger quantities of blood, however since design requirements dictate that only 100 µL of blood be used, an aspiration is a much faster and more efficient introduction system. In order to better understand the amount of pressure needed for this form of aspiration the team created a simplified mathematical model of the system shown below.

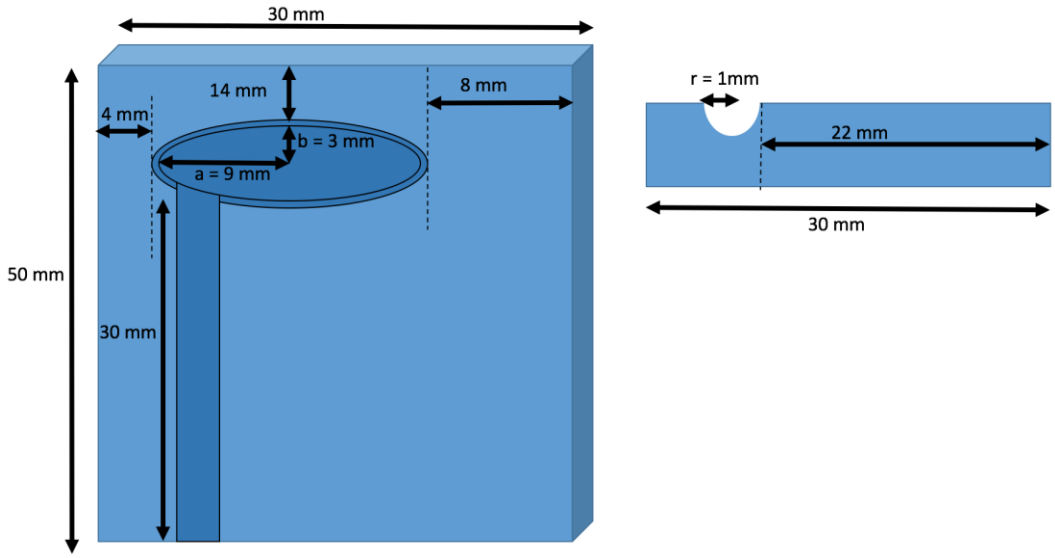

Figure 18: Dimensions Channels and Reservoir, Design 2.0

The following equations describe the formulations used to find the necessary pressure for the reservoir to pull blood through a channel of a certain width and diameter capable of housing IL's electrolytic sensors. ρ is the average density of blood, *g* is the gravitational constant, and *h* is the channel length necessary to pull blood into both the capillary tube and the reservoir. Using *Equation 5,* we were able to find the pressure necessary to pull 100 µL of blood through the initial capillary tube and into the reservoir. The results are as follows in the calculations below.

$$\rho = 1.06 \frac{g}{cm^3} = 1.06 \cdot 10^3 \frac{kg}{m^3} \quad (5)$$

$$g = 9.89 \frac{m}{s^2}$$

$$h = 30mm = 0.03m$$

Pressure to fill capillary was then found using the density, gravitational constant, and height of the liquid using *Equation 6*.

$$P_{capillary} = \rho g h \quad (6)$$

$$= \left(1.06 \cdot 10^3 \frac{kg}{m^3}\right)\left(9.98 \frac{m}{s^2}\right)(0.03m)$$

$$P_{capillary} = 317.36\ Nm$$

Pressure to fill reservoir was also found using the area of the channel, fluid velocity, and distance. In *Equation 7*, *a* is the longer diameter and *b* is the shorter diameter of the oval reservoir.

$$A_{reservoir} = Area\ of\ Oval = \pi ab \quad (7)$$

$$= \pi(9mm)(3mm) = \pi(0.09m)(0.03m)$$

$$A_{reservoir} = 8.48 \cdot 10^{-5}\ m^2$$

$$V_{fluid} = 100\ \mu L = 100\ mm^3 = 1.0\ 10^{-7} m^3$$

$$Distance_{fluid\ in\ reservoir} = \frac{V_{fluid}}{A_{reservoir}} = 1.0\frac{10^{-7}m^3}{8.48\cdot10^{-5}\ m^2} \quad (8)$$

$$Distance_{fluid\ in\ reservoir} = 1.18 \cdot 10^{-3}\ m$$

$$P_{reservoir} = \rho gh = \left(1.06 \cdot 10^3\ \frac{kg}{m^3}\right)\left(9.98\frac{m}{s^2}\right)(1.18 \cdot 10^{-3}\ m) \quad (9)$$

$$P_{reservoir} = 12.48\ Nm$$

Total Pressure needed can be found by summing the pressure needed for the capillary tube as well as that for the reservoir.

$$P_{total} = P_{capillary} + P_{reservoir} \quad (10)$$

$$= 317.36\ Nm + 12.48\ Nm$$

$$P_{total} = 329.84\ Nm \approx 0.0480\ psi$$

From these calculations, it is evident that the amount of pressure needed exceeds that which is feasible based on the equipment available. The list of potential points of this device are numerous: the silicon micro-valve, the complete seal of the base plate to the cover, and the significant amount of pressure needed to draw in the fluid. The persistence of these design flaws across iterations made clear that fabricating this type of cartridge would be both costly and time consuming. This lead the team to consider with other designs.

4.3.2 Pump and Capillary Action

Pumps are a common source for creating a pressure differentials, and is actually the current method IL uses in their GEM blood gas analyzer line. With this in mind, the pump offers an inherent advantage in that IL's familiarity with pumps will allow them to optimize and fabricate our rudimentary pump design faster and with better accuracy. We thus investigated the plausibility of using two specific pump types: peristaltic and solenoid.

4.3.2.1 Peristaltic Pump Design

Peristaltic pumps are used to pump a variety of fluids using positive displacement. These pumps have a set of "rollers" fitted to the ends of a rotating disc, which periodically compress a length of tubing, thereby forcing the fluid along by the positive displacement generated [51]. This causes suction to occur on the inlet side while the outlet tube enables the fluid to flow out [52]. These pumps are typically used in pharmaceutical and semiconductor applications, dialysis systems, and heart-lung machines [53]. The fluid flow is controlled by a stepper motor rotating the axel of the disc, which in turn produces a high flow rate resolution and constant laminar flow [54]. Laminar flow is ideal in this situation as it prevents mechanical deformation that may denature the red blood cells (RBCs).

A micro-peristaltic pump was attached to the hand-held device model, shown in *Figure 19*, to exhibit what it would ideally look like on the chip. The figure shows how the rotor compresses the

silicon tubing at the top left, moving the fluid through the cartridge. The tubing would interface with the main cartridge body using hollow metal pins.

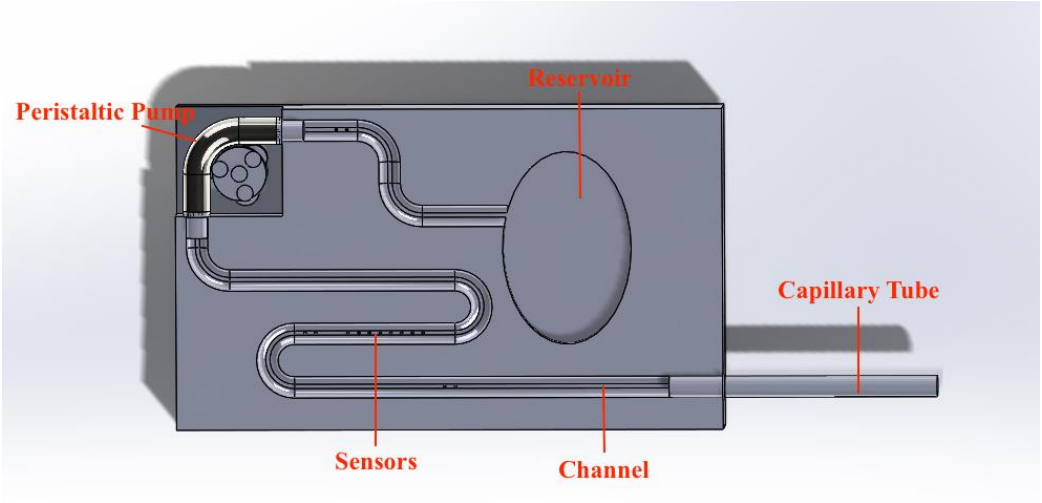

Figure 19: SOLIDWORKS Peristaltic Pump Compartments Top View.

In this preliminary design, the process begins with the suction of the blood through the capillary tube, which then leads to the entrance of the microfluidic channel. Positioning sensors are placed on the channel length before the first channel curve, immediately before the analytic sensors, and finally right after the silicone tubing for tracking fluid movement and prevent over drawing. The analytical sensors are placed after the first channel bend to allow enough time for the sample to heat up. Reagents sitting on the sensors will be moved to the reservoir in order to allow space for the sample. The rotor axel would be attached to a miniaturized motor that would spin at a speed corresponding with the desired flow rate.

As mentioned, IL has had the most experience designing, fabricating, and implementing peristaltic pumps since they are used in their GEM blood analyzer series. This advantage compounded with the fact that they are easily manipulated achieve a steady but constant flow make them a highly regarded design. However, due to the need of creating a leak proof seal between the silicon tubing and metal pins and of printing the rotor assemble on every cartridge, the manufacturability of this design is very improbable.

4.3.2.2 Solenoid Pumps

Solenoid pumps are used to pump air and a variety of fluids by creating suction using a diaphragm composed of a spring and a piston. Coils outside the piston utilizes an electric charge to control the spring that in turn allows for compression and stretching. This action allows for the piston to move upwards (suction position) and downwards (ejection position) [55]. Solenoid pumps, similarly to peristaltic pumps, allow for high flow rate resolution and ever near continuous flow despite the pumping action. However, high pumping speeds are prone to inducing turbulent flow. Additionally, the diaphragm necessary for this pump action must have direct contact with the fluid inside of the channel to allow for enough pressure to build.

A micro-solenoid pump was attached to the microfluidic chip through SOLIDWORKS to exhibit what it would look like as shown in *Figure 20*.

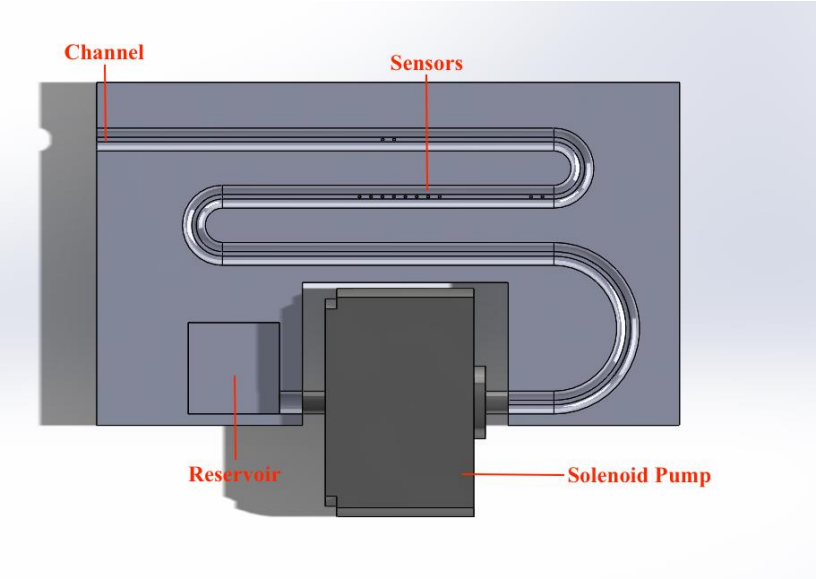

Figure 20: SOLIDWORKS Solenoid Pump Compartments Top View.

Similar to the previous peristaltic pump, positioning sensors are placed on the channel length before the first channel curve, immediately before the analytic sensors, and finally right after the silicone tubing for tracking fluid movement and prevent over drawing. At the solenoid pump, fluid ejection through the outlet occurs when the piston moves downwards, pushing down on the fluid. As the fluid is ejected, it moves through the outlet and into the reservoir and a fresh section of either reagent or blood is pushed into the pump, waiting for the process to repeat again. A major weakness to this design is that it must operate at low flowrates to allow for laminar flow to occur. Additionally, the choppiness of the flow rate due to the piston movement may cause irreversible damage to the blood sample. The major concern is the fact that the piston component requires direct contact with the fluid, compromising the biohazard containment of the sample. This inherent flaw with this design led us to not select the solenoid pump as a potential fluid movement mechanism.

4.3.3 Microneedle Patch

Microneedles are a minimally invasive, micro-sized device that can disrupt the top layer of the skin [56-58] . Used for transdermal drug delivery and diagnostic sensors, the field of microneedle devices is still growing and there are new biomedical devices currently being developed. This design utilizes an elongated version of these needles to draw blood directly from the capillaries for analysis.

The microneedle patch design is an array of microneedles on an adhesive patch that can be placed anywhere on the body. It would pull in capillary blood from the skin and collect it in a reservoir at the end of the needles and on the patch. The IL sensors would be placed in that reservoir and would perform the blood gas analysis. On the patch would be an RFID tag or other means of wireless communication which would send the blood gas data to a handheld reader. The microneedle patch can be designed for multiple readings on one patient while still being disposable. *Figure 21* shows of

a magnified view of what the hollow needles would look like while *Figure 22* shows the schematic of a single theoretical microneedle with blood collection reservoir and sensors.

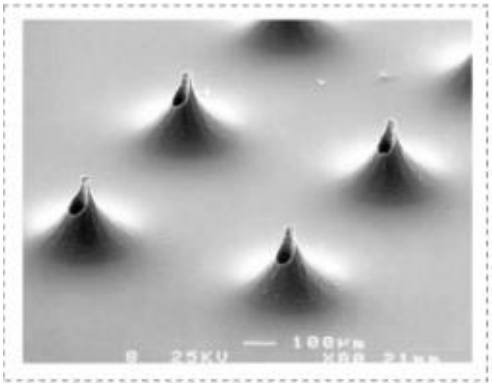

Figure 21: Microneedle Array with Needles of 100 µL

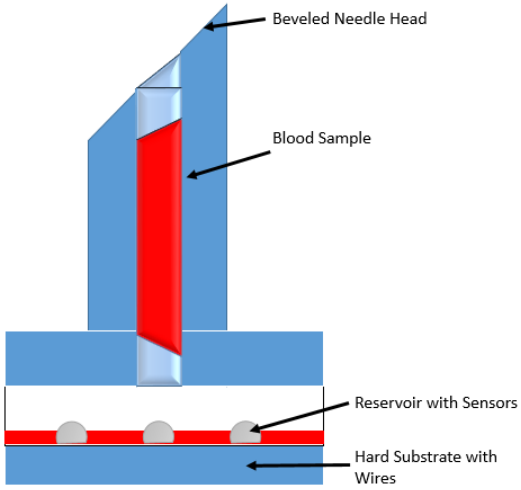

Figure 22: Microneedle Design Schematic

4.3.3.1 Microneedle Current Technology and Uses

Microneedles have been fabricated with lengths of 0.4mm – 2.0mm and have been studied to be less painful and 26-gage hypodermic needles [59]. The microneedle patches can also be used by patients at home in a direct delivery method [60]. This field is in increasing in development due to the decreased pain, ease of application, and lower cost for patients. Microneedles are currently used for transdermal drug delivery and diagnostic sensors.

The most common use of microneedle technology is for drug delivery. Microneedles can be designed to have a hollow profile to allow flow of biotherapeutics or vaccines into the epidermal layer of the skin. For this method, there is at least one reservoir containing the drug. Typically, the devices use a plunger as means to compress the reservoir and move the drug from the reservoir to the destination [61]. Other microneedles are made to dissolve and deliver the drug after penetrating the

skin [60]. Another method coats the microneedles in the drug and then have them puncture the skin for delivery. The microneedle drug delivery method is not used for many drugs due to the poor permeability of the skin that acts as a barrier to most drugs [61].

A recent development in the use of microneedle technology is glucose detection and observation for diabetic patients. The transdermal monitoring technique is useful for time sensitive clinical information. These microneedles are typically 200 µm long and analyzes glucose in the interstitial fluid [57, 62]. The microneedle patches combine electrochemical sensors and drug-release actuators [56]. The process works as follows: passive diffusion moves the interstitial fluid from the body, i.e. a finger, to the microneedle array where it is mixed with a buffer solution and moves to the sensors placed downstream from the microneedle array [57]. There can be ex vitro or in vivo monitoring based on sensor construction and placement. There is no current POC diagnostic applications that are based on microneedles.

4.3.3.2 Design Specifications

There are many considerations for this design, specifically pertaining to the dimensions of the microneedle: diameter, length, array profile, and sensor placement.

The diameter of the microneedles must be at least 10 µm to allow blood cells to pass without cause of damage or lysis. This requirement is directly taken from the design requirements: the device shall maintain blood sample integrity (reduce hemolysis) and complete analysis on whole blood. The diameter must be an appropriate size to allow red blood cells to pass without rupturing. However, sharper needle tip (smaller in dimension) reduce the size of puncture wounds [62]. Therefore, the microneedle shaft diameter must be thin enough to allow the least amount of damage on the patient and skin but wide enough to allow the least amount of damage on blood cells.

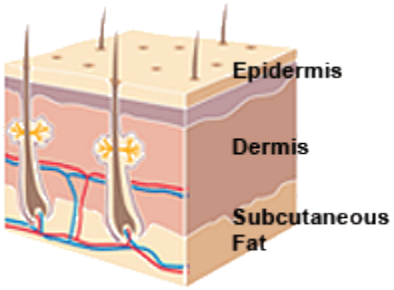

Figure 23: Layers of the Skin [56]

The patch would be placed on the skin to allow for the microneedles to puncture through the skin and analyze the blood. In order to reach the vessels, the needles must reach the lower level of the dermis, as shown in *Figure 23*. The epidermis is 10 – 20 µm deep while the dermal epidermal junction is 80 µm. The dermis is 1000 µm deep in total where the lower part starts half way through, or 500 µm into the dermis level [63]. The current observation of glucose only requires the microneedles to reach the interstitial fluid of the skin in the epidermis layer. Our project is to develop a blood gas analysis device therefore, the microneedles must be of a longer length to reach at least the capillary beds in the lower dermis level. The minimum length of the needles is between 1-1.5 mm. According to research on the pain effects of microneedle designs, the longer the microneedle length, the more painful it is for the patient because the increased surface area of the needles exposes more nerve, causing pain receptor excitation [59].

The glucose monitoring microneedles are capable of pulling up to 10 μL of interstitial fluids per needle [57]. If each microneedle would be able to pull approximately 10 μL of blood through, the design would need 10 microneedles to be able to collect no more than 100 μL of blood. Further testing would be required to understand the movement and speed of blood through the microneedles and its effects on the volume. Another note is that the number of microneedles had little effect on pain [59]. However, the design would still aim to have 10 microneedles on the patch.

Current technology would dictate the sensor layout of the blood analysis design and we would place the sensors in the reservoir at the end of the microneedles, on the patch. The patch would need to be big enough to fit 10 microneedles, the reservoir, the sensor reagents, and a component similar to an RFID tag. If this design would be used for multiple readings for one use, then the patch would need a larger waste reservoir based on number of readings per patch.

This design would fit many of IL's user needs such as analyzing whole blood, fitting in the palm of the user's hand, being held in any orientation, performing analysis at body temperature, and have a disposable sample component. However, due to the innovative nature of this product, it was uncertain how compliant this design would be with requirements such as: analysis in less than 3 minutes and position blood over sensors repeatedly. There are still other downfalls such as manufacturing abilities, eliminating contamination (from the skin), and integration of IL sensors. The team decided not to pursue this design because there are many uncertainties and difficulties with testing, manufacturing, and supplies.

4.1.3.3 Design Analysis

This design has many benefits. Microneedles are less painful, easier to understand, and cheaper for patients. The design would be a minimally invasive way of collecting blood gas analysis data while also being close to accurate, real-time analysis by performing direct contact collection from the patient. This is useful for time-sensitive information. It allows for less time between blood collection and blood analysis, minimizing the amount of air contamination that could skew blood gas values. It is also disposable and prevents spread of blood borne pathogens through reusing needles.

The design also fits a number of the user needs. With proper specifications, the microneedle array would be able to complete analysis on whole blood without damaging the cells. I can be designed small enough to pull in the right blood sample size while still being small, disposable, and limiting operator exposure to biohazard. This design leaves room for future improvements on the design that cannot be met within the time scope of the project. This involves modeling the handheld reading device to receive the RFID data from the patch and convey test status and results to the user.

However, there are still many disadvantages and concerns for this design. The length of the microneedles would only reach the capillary bed, only to perform blood gas analysis on capillary blood rather than arterial blood. The analysis of capillary blood would be different than arterial blood due to the blood gas physiology in the capillary bed, thus skewing the analysis algorithms IL has already developed. Another downside is that IL sensors require a ridge surface. The patch would be flexible in order to stick and remain comfortably on the patient. It would be difficult to integrate the sensors on the patch by either redesigning the sensors to work on any surface or design the patch to be more ridge.

Another barrier to microneedles is the stratum corneum layer of the skin. This is the outer layer of the skin and is covered in dust, dead skin cells, and other debris. When the microneedles

puncture the skin debris from the stratum corneum blocks the microneedles and can disruption in the system. This would not maintain blood sample integrity. This is a current problem for microneedles with monitoring and sensing purposes [57].

Our team lacked the fabrication machinery necessary for creating hollow microneedles, such as a reactive ion etching machine, integrated lithographic molding, deep X-ray photolithography, or laser micromachining. In addition, the cost of validating our needle formation through atomic force microscopy would also be time consuming and costly. In this sense, we are limited in resources.

In summary, due to the limitations of time and resources, as well as the design having many modes of failure, this concept was not pursued any further. The monumental advantages of this design included little to no discomfort to the patient while minimizing the cartridge that required no moving parts. However, we would not be able to manufacture and test in the time frame given.

4.3.4 Compressed Air

In most designs for fluid movement, we created a negative pressure to act as a linear drawing mechanism (a pump) to pull fluid into our system and position it. Compressed air entering an air cylinder (also known as a pneumatic cylinder) is capable of creating a linear displacement using a piston to allow for the drawing of fluid. All variations of this design depend on design modifications of the cylinder's barrel size, piston length and disc, and rate of pressure input and release.

There exists two major types of cylinders: single and double acting. In single acting, a singular aperture, in this case the barrel, allows for air to enter the piston and push the piston disc toward a positive displacement. Once this piston disc is fully displaced a spring re-actuates the piston to its original neutral position. A single acting compressed air motor the piston is driven down by the introduction of air into a chamber and is returned to its starting position by a spring.

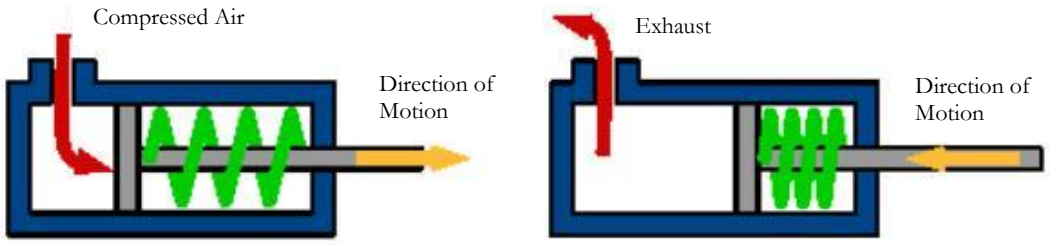

Figure 24: Schematic of a single acting air cylinder

Consequently, a double acting air cylinder uses compressed air to move the piston disc in both directions; both to displace it and to return it to its original neutral position. *Figure 25* show

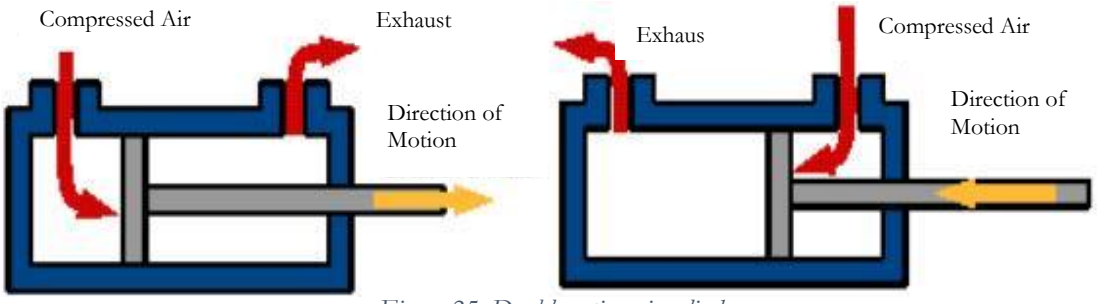

Figure 25: Double acting air cylinder

the design and the mechanics of a double acting air cylinder. This method however, requires more compressed air to operate over time.

If our team were to pursue this potential design, we would most likely acquire a reverse acting or air retract cylinder, which resembles the single acting cylinder except that the default piston rod position is already drawn out. This would be mounted inside the hand held module and interface with the cartridge's metal port. Once air enters the cylinder, the rod is pulled back into the barrel and a spring would return the rod to its extended position afterwards. Once the cartridge has been connected to the hand held system, a microprocessor would allow for compressed gas to enter the cylinder and pull the piston disc back in order to pull liquid in. Compressed air flow can be controlled electronically by either attaching a solenoid pump to the air hose or flow control fittings at the air ports.

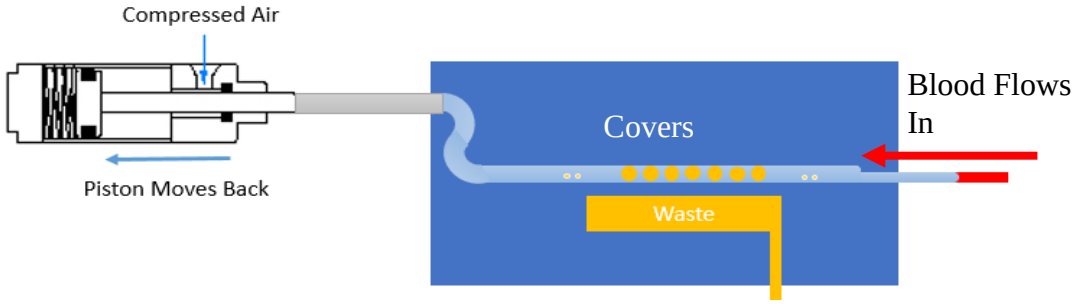

Figure 26: Compressed Air Cylinder Interfacing with the Microfluidic Cartridge

Some of the major advantages of this proposed system is that it offers a smooth draw of fluid without any fluctuations in flow rate. In addition, there is no leakage of fluid since here the operating mechanism is gas, and the system would be compact with the air compressed tank being the limiting factor. The controlling systems for these cylinders can be sensitive enough to allow for very accurate fluid drawing, and during mid test change the direction of fluid draw. Current market cylinders can vary drastically in size, the smallest being found during our research to include bore sizes of 1/4", 3/8", and ½" and can come in both single and double acting models, air reverse and air forward [64]. By varying stroke (how far the piston rod extends), and bore (cylinder diameter and thus the main factor in controlling pull force) in addition to air pressure and spring tension, our team would be able to calibrate a very precise system capable of positioning 100 µL of blood over the sensors [65]. The price range of a single air retracting cylinder of bore size 7/16" with rod length of 1/2" was approximately mid $30.00 to $60.00 [66]. However, compressed air build up in either the cylinder barrel or the compressed air source poses formidable risks to the operator. In addition, the device would need replacement compressed air canisters after a certain amount of uses, increasing long term operation costs already set by the disposable microfluidic cartridge. The other fail method (air leakage at any point in the system) would not pose as dire a threat to the operator, and effectively would diminish the strength of fluid flow only. A system of locks would be implemented to prevent buildup of air, while acquired a sensor ready air cylinder would allow it to provide a negative feedback loop to prevent over or under flow as well as flow direction.

4.3.5 Syringe Pump

Born from the combination of the advantages of the active pumping and differential pressure designs, the syringe pump utilizes a 1 ml syringe controlled by a stepper motor to create negative pressure for fluid movement. This design has the active feedback for accurate fluid positioning,

negative pressure for fluid movement without the single point of failure in a membrane, the ability to aspirate from containers, and a simple, cheap cartridge design. The cartridge could easily prototypes accurately and quickly with the help of a machine shop while the materials for the pulling plate mechanism could easily be found in a hardware store. Together, the system was easy to assemble at a low cost. Not only is this design the cheapest in terms of overall disposable cartridge design, but it also has the simplest circuit design for the handheld device. The stepper motor is easily programmable, extremely accurate for positioning, and allows for many different insights into the fluid within the cartridge through the use of positioning sensors. These sensors provide active feedback that monitor fluid positioning, fluid velocity, and after proper testing could estimate the viscosity and hemocrit level of the blood. Overall fluid movement is based on negative pressure and removed any form of piston or rotor from the cartridge design, which decreased total cost per unit, as well as the necessity to operate at a specific orientation. The cartridge design is shown in *Figure 27* as well as the distinction of the inlet and outlet and the attachment of the pump system shown in *Figure 28*.

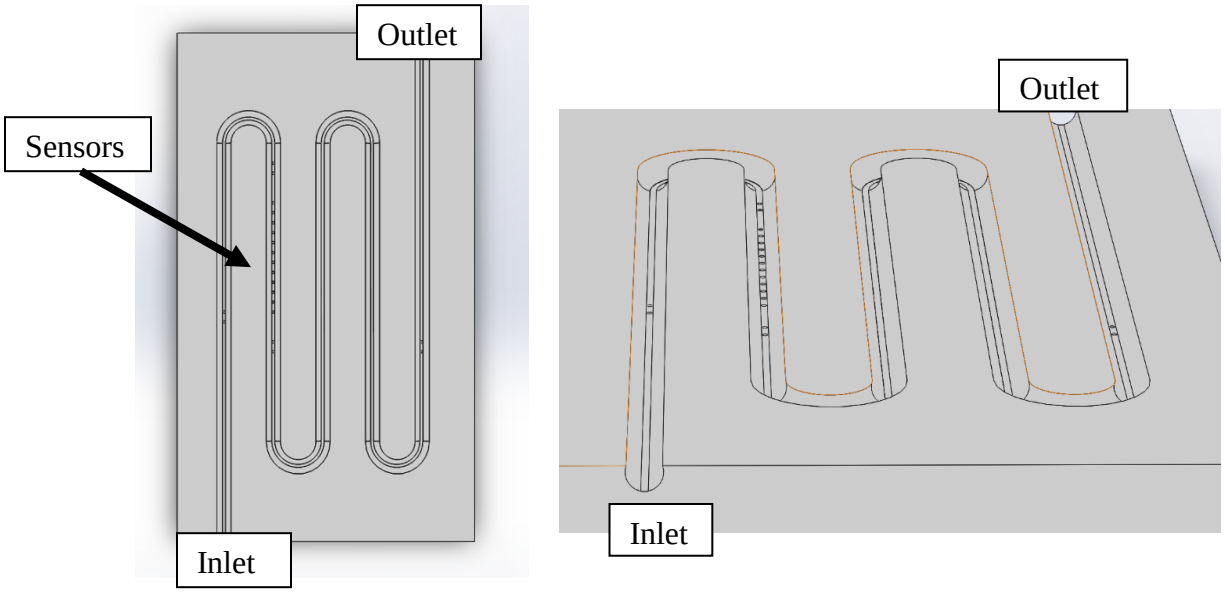

Figure 27: Syringe Pump Cartridge Design

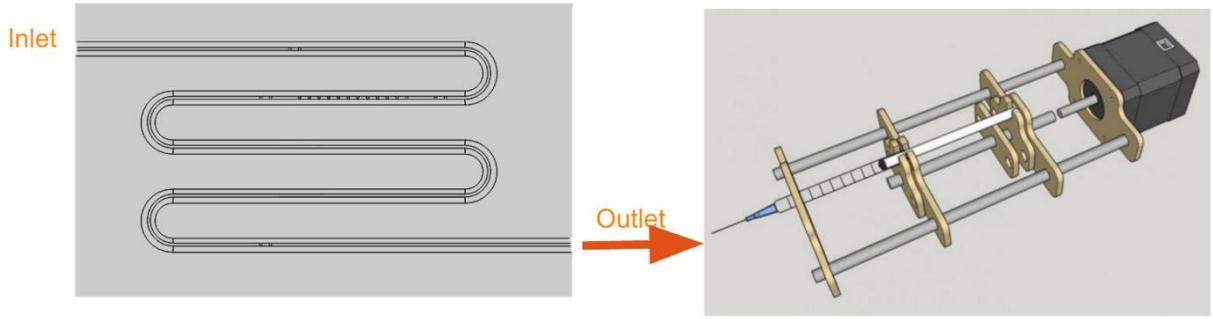

Figure 28: Syringe Pump Cartridge Design, Inlet and Outlet and Pump Connection

4.4 Fluid Modeling

Computational Fluid Dynamics (CFD) is the area of fluid behavioral modeling simulation through the use of numerous general algorithms (i.e. using the Navier-Stokes equations to use the finite volume technique) that are implemented via various software. In this section, we discuss the programs that we initially pursued and our final selection of SOLIDWORKS Fluid Simulation as the main source for our fluid modeling needs.

4.4.1 MATLAB

MATLAB, or MATrix LABoratories, allows users to perform matrix based computations. This program offers many toolboxes and visualization software for a variety purposes from signals processing to computational finance, and even computer design. Due to its versatility and computational strength as well as its ease of use, our team saw an opportunity to potential implement fluid modeling for short developmental lengths using this software. With this in mind, IL has used MATLAB to implement models of fluid flow through a combination of differential equations that equate components of the system to circuitry as dictated by the electronic – hydraulic analogy. However, due the complexity of IL's current code as well as their need for several very specific libraries not available to us, we chose not to pursue MATLAB as our fluid modeling software

4.4.2 ANSYS

Another potential program that was suggested to us was ANSYS CFD. ANSYS itself creates many simulation tools ranging from finite element to structural analysis. ANSYS CFD specifically allows engineers to perform incredibly accurate computational fluid dynamic simulations within a closed environment and would allow for specification of nonlinear material properties such as plasticity. Thus was of interest to our team. However, we found that the instability of the software on our remote server, as well as the general use for ANSYS was for more complex fluid modeling proved that it did not suite our needs.

4.4.3 Comsol

Comsol software can be used for multiphysics simulation, with a specific software for microfluidic device simulation. It can be used with lab-on-a-cartridge devices similar to our design concepts and take into account many microscale effects such as surface tension forces, capillary forces, diffusion, and chemical transportation [67]. The team got into contact with Worcester Polytechnic Institute Professor Brian Savilonis from the Mechanical Engineering department with research in biological fluid flow and Professor Dirk Albrecht from the Biomedical Engineering working with biomedical micro-technology. Both have experience with Comsol and gave insight on the software. Professor Savilonis recommended Comsol for direct inputs with simple geometry and can get a good constitutive model for blood even though it will not take into account the actual red blood cell size [68]. Professor Albrecht said that the software is good for local modeling but can be overwhelming for entire microfluidic networks [69]. Our blood analysis cartridge concepts are more complicated and larger than simple geometric shapes and local microfluidic networks. Based on this advice, Comsol was shown to not be an appropriate modelling software for our system.

4.4.4 SOLIDWORKS

SoldWorks is a wildly used solid modeling computer-aided design (CAD) program published by Dassault Systems. It can created 3 dimensional models and assemblies via a parametric feature-based approach, and thus our team has used it to draft our microfluidic cartridges for visualization and 3D printing. With this in mind, we found that the Flow Simulation add on in the program directly interfaced with our designs with only minor modification (i.e. addling lids to create a water tight

enclosure). In addition, SOLIDWORKS Flow Simulation also contained preprogrammed values for specific non-Newtonian fluids including blood. Due to its ease of use, ready availability through WPI, and seamless interfacing with our current models, we chose SOLIDWORKS as our final CFD.

4.4.5 Modeling Software Selection

With the decision to move forward with the SOLIDWORKS modeling software the team tested the syringe pump cartridge design. The preliminary results are shown below in *Figure 29* through *Figure 33*.

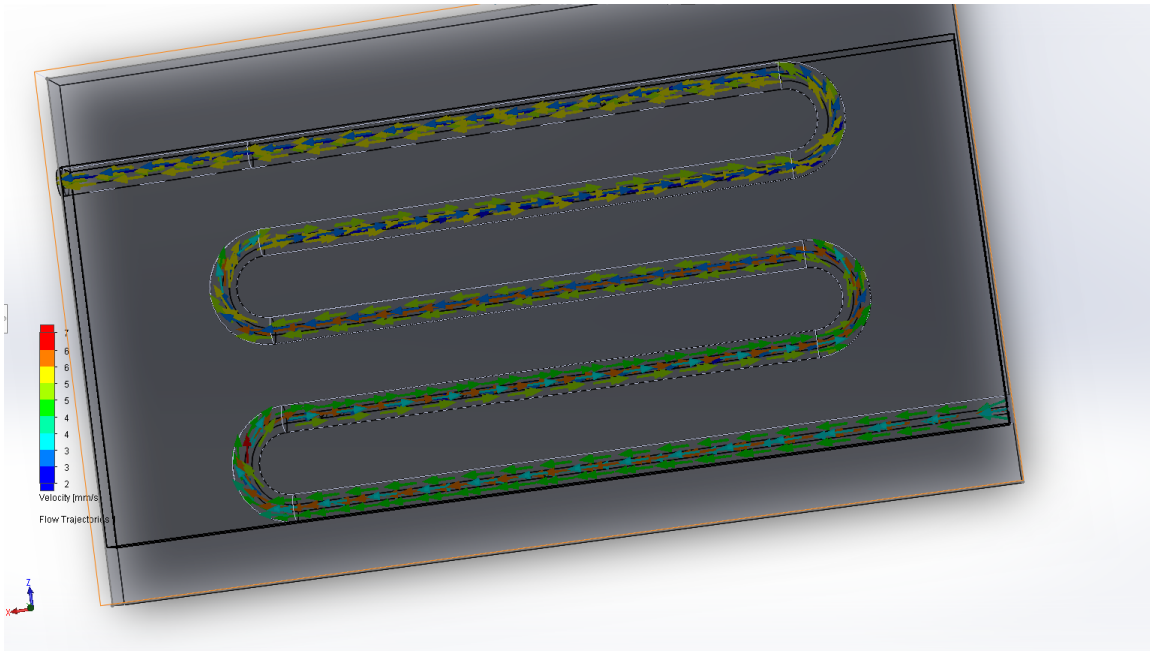

Figure 29: Fluid Trajectory Map of the Syringe Pump Model

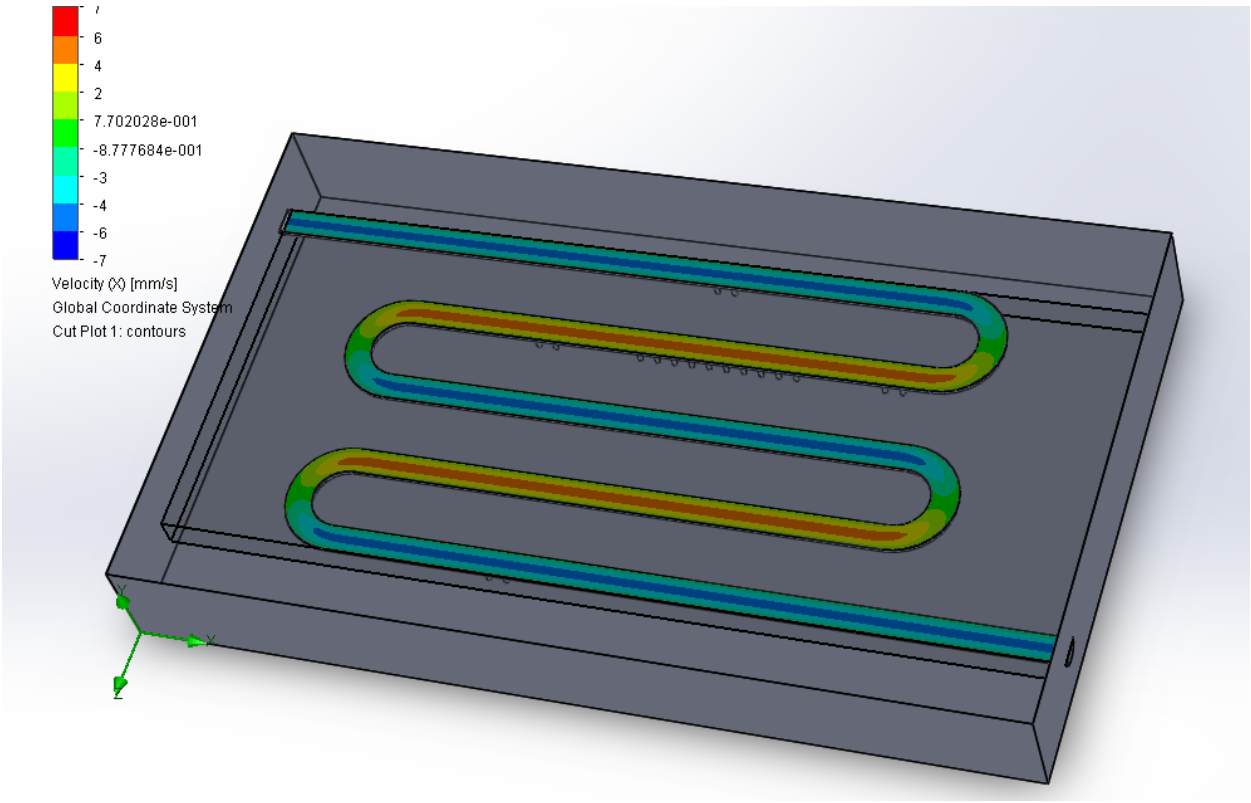

Figure 30: Fluid Velocity Map of the Syringe Pump Model

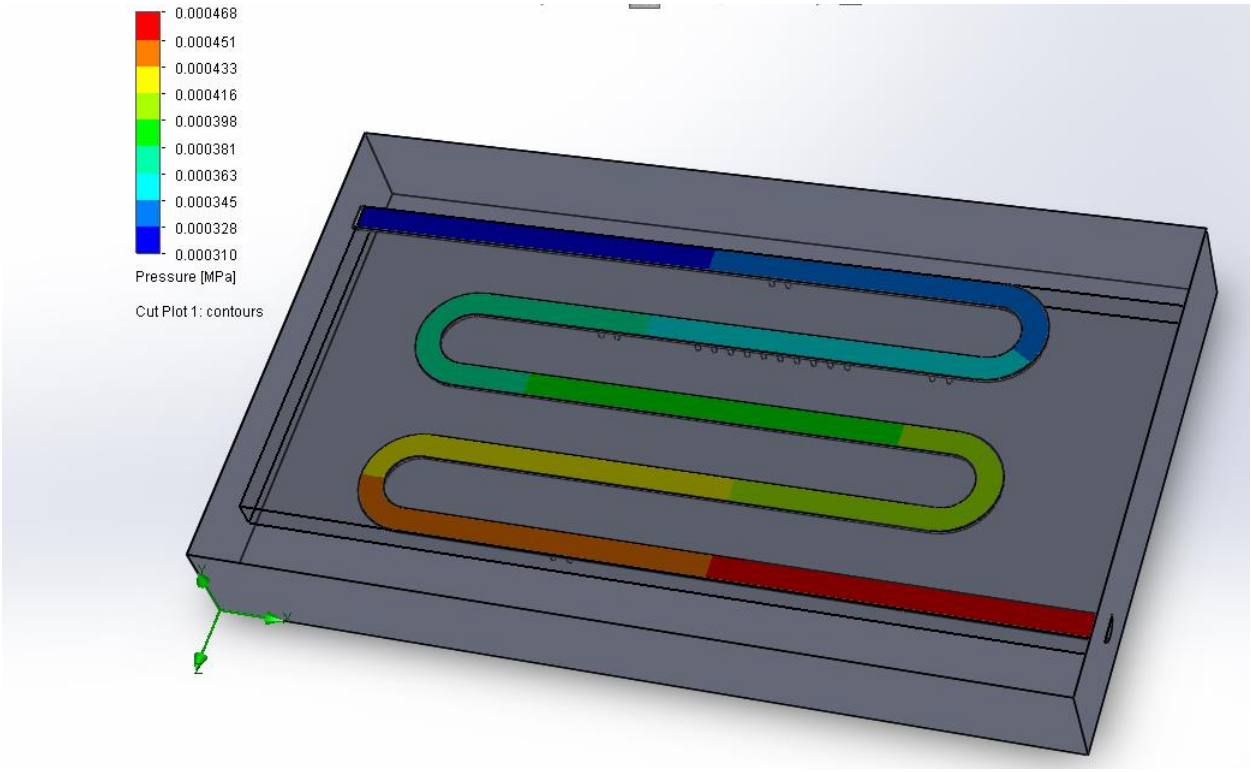

Figure 31: Pressure Map of the Syringe Pump Model

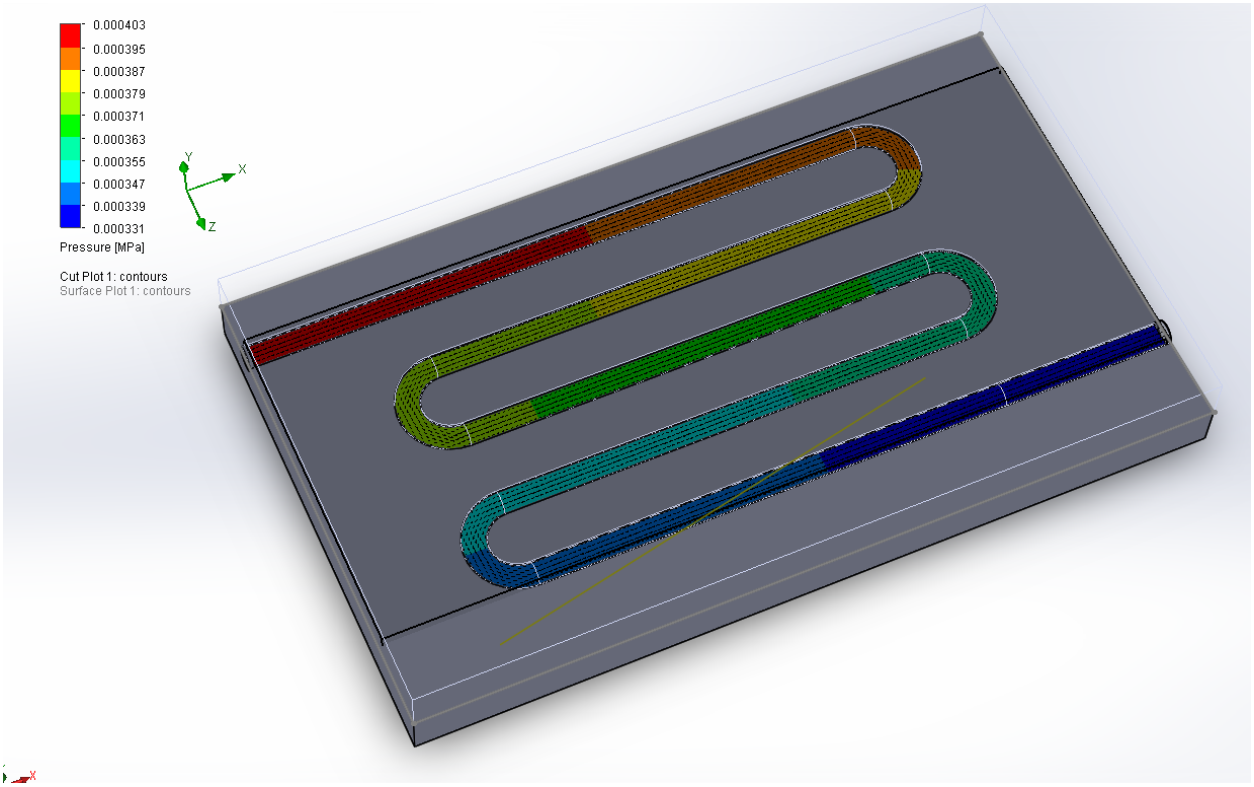

Figure 32: Fluid Velocity Map without Cover

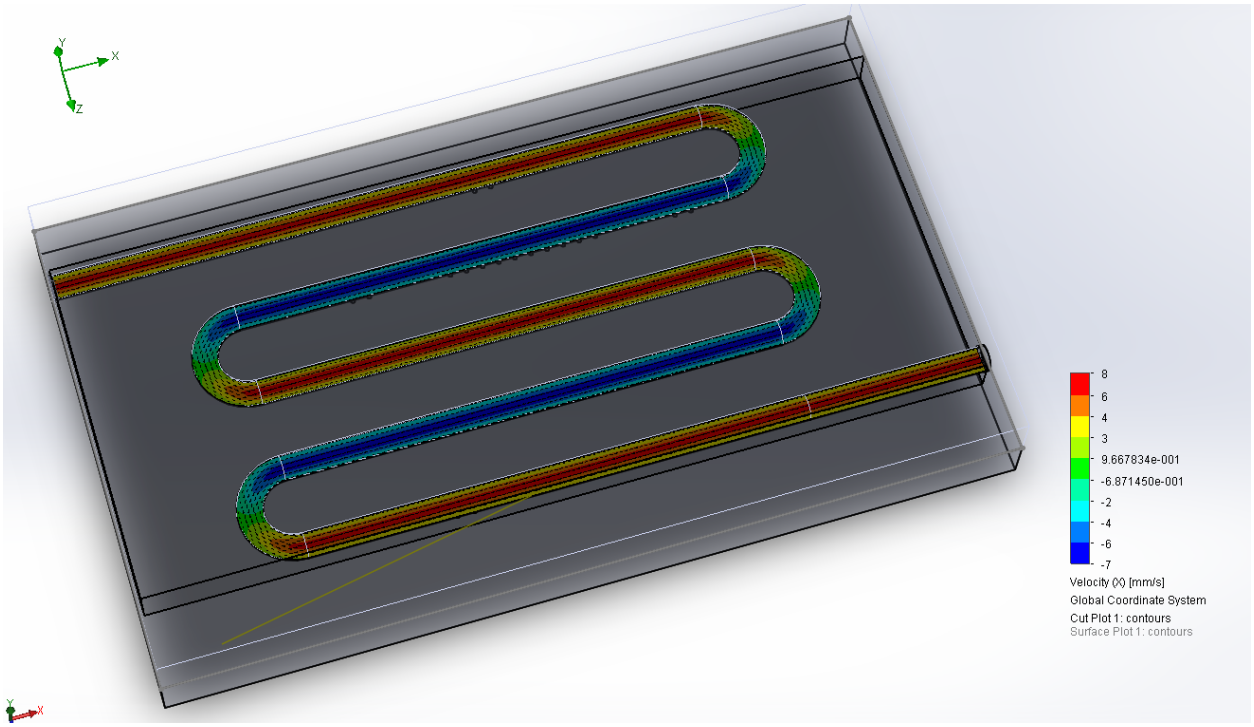

Figure 33: Fluid Velocity Map with Cover

Throughout the design processing CFD acted as our predictor to potential design flaws that may compromise the integrity of our sample. For instance areas where our simulation indicated high areas

of pressure or velocity may suggest potential areas in our cartridge that may induce clotting or hemolysis. Simulations were run on every cartridge design before fabrication and proved to be a very useful tool for design validation.

4.5 Final Design Selection

The first step in selecting our final design was by creating a Pugh Matrix to quantitatively compare our 3 most viable concepts which were the direct draw through differential pressure, pumps (peristaltic and solenoid), and the microneedle array. We gave the 18 user needs a ranking from 1 to 5, 1 being the least important and 5 being the most important pertaining to the functionality of our final device. The results are shown in *Table 4*.

Table 4: Pugh Matrix of Design Concepts

User Needs	Weights	Direct Draw		Pump		Microneedles	
		Score	Weighted	Score	Weighted	Score	Weighted
The device shall complete analysis in less than 3 minutes	5	4	20	4	20	3	15
The device shall maintain blood sample integrity (eliminate contamination air and reagent, reduce hemolysis)	5	5	25	4	20	3	15
The device shall position the patient blood sample repeatably over blood sensors	5	3	15	3.5	17.5	3	15
The device shall complete analysis on whole blood	4	4	16	5	20	5	20
The device shall complete analysis with a blood sample no larger than 100 uL	4	3	12	4	16	4	16
The device shall fit in the palm of a users hand	4	5	20	4	16	4	16
The device shall measure Chloride, Potassium, Calcium, pO2, Sodium, pH, pCO2, using existing IL sensor technology	4	5	20	5	20	3	12
The device conveys current test status to user	3	3	9	5	15	4	12
The device shall be able to aspirate blood from a syringe	3	1	3	5	15	0	0
The device shall have a disposable single use sample component	3	5	15	5	15	5	15
The device shall limit operator exposure to biohazard	3	4	12	3	9	4	12
The disposable component of the device shall cost less than 1/10 of typical analyte/test reimbursement	3	4	12	5	15	3	9
The disposable component shall interface with the device electronics to support sensor function	3	4	12	5	15	5	15
The device shall be RoHs compliant	2	5	10	5	10	5	10
The device shall comply with IEC 60601	2	5	10	5	10	5	10
The device shall perform analysis at body temperature (37.4 +/- 0.3 C)	2	5	10	3	6	4	8
The device leverages open source electronic platforms such as Arduino, Raspberry Pi, Beaglebone Micro controllers	1	0	0	1	1	4	4
The device shall operate in any handheld orientation	1	2	2	4	4	4	4
Total:	**285**		**223**		**244.5**		**208**

Based on these scores, the microneedle design had the lowest score of 208 out of 305. Although the microneedles offers many benefits including direct sensing, and minimal discomfort, there were more uncertainties that both WPI and IL's facilities were incapable of compensating for with our limited resources both in design and fabrication. Additionally, the in vivo preliminary porcine model required for initial testing posed a many issues both financially and time wise. This concept also lacked the hard substrate necessary for IL's sensors to rest on, an inherent design flaw. Finally, the manufacturing of the microneedles involves fabrication machinery that are not available to the team, such as an integrated lithographic molding for fabrication as well as atomic force microscopy (AFM) for size validation. In addition, the cost of validating our needle formation through atomic force microscopy would also be time consuming and costly. The team was limited in time, feasibility, availability, and resources for manufacturing the microneedles and enacting the test procedures. Therefore, we did not continue on with this design.

The direct draw method had a score of 233 while the pump designs had a score of 244.5. Because both scores are similar to each other, second iteration of both designs were created with more weight added to user needs more necessary for device functionality. Although the direct draw method uses vacuum created differential pressure, another method was created using compressed air to pull the fluid through by creating a pressure differential. However, compressed air can be highly dangerous to the user, potentially causing a pressure build up leading to an explosion and spray of biohazardous (i.e. blood or reagent) fluid onto the user. We chose to not pursue this design due to this high risk factor.

The major drawback of the differential pressure design was that it relied heavily on a rubber septum as a cylindrical protrusion compatible with phlebotomist equipment in order to initiate the differential pressure and draw blood in. The cartridge could receive blood from syringe or direct draw from the patient but not aspirate blood, the easiest loading mechanism for phlebotomist. Therefore in the second iteration, we removed the rubber septum and replaced it with a silicon micro-value. This allowed for blood direct withdrawal from a syringe and an addition of aspiration, making the design more universal for receiving blood. The design also was compatible with current IL desktop technologies and can be manufactured the same way as their current cartridges. These are advantages persuaded the team to continue refining this design.

The differential pressure design had major drawbacks, and so was pass over in favor of a more reliable design. For instance, this concept required high accuracy during the manufacturing stage in order to insure the correct amount of pressure is sealed in each cartridge. Furthermore, the device may be prone to air leakage over time, reducing the cartridge shelf life which is an important device aspect that must rival if not exceed the standard of the competitor's products. The pump designs, specifically peristaltic pumps, also have more reliable and accurate fluid movement control with the potential for an active feedback mechanism. Fluid movement is important to position the blood over the sensors, and therefore the team chose to continue on with a pump design rather than the differential pressure direct draw design.

With the pump designs, we have three options: peristaltic, solenoid, and syringe pump. The solenoid consumes less power and is more durable to wear and tear compared to the peristaltic pump. But the solenoid pumps need to come in contact with the fluid, therefore each new cartridge requires a new pump. Even though the solenoid pump is cheaper than the peristaltic pump and stepper motor, the design itself is more expensive because the pump must be disposed of after every use. It is unreasonable compared to the peristaltic and syringe pump which can place the pumps in one reusable handheld device rather than the disposable cartridge. The team decided not to pursue the solenoid pump and focus on narrowing down between the peristaltic pump and the syringe pump.

We created a second Pugh matrix for our final two design concepts: the peristaltic and syringe pumps in their later iterations as seen in *Table 5*.

Table 5: Pugh Matrix for Final Two Design Concepts

User Needs	Weights	Peristaltic Pump		Syringe Pump	
		Score	Weighted	Score	Weighted
The device shall complete analysis in less than 3 minutes	5	5	25	5	25
The device shall maintain blood sample integrity (eliminate contamination air and reagent, reduce hemolysis)	5	5	25	5	25
The device shall position the patient blood sample repeatably over blood sensors	5	5	25	5	25
The device shall complete analysis on whole blood	4	5	20	5	20
The device shall complete analysis with a blood sample no larger than 100 uL	4	5	20	5	20
The device shall fit in the palm of a user's hand	4	5	20	5	20
The device shall measure Chloride, Potassium, Calcium, pO2, Sodium, pH, pCO2, using existing IL sensor technology	4	5	20	5	20
The device conveys current test status to user	3	5	15	5	15
The device shall be able to aspirate blood from a syringe	3	5	15	4	12
The device shall have a disposable single use sample component	3	4	12	5	15
The device shall limit operator exposure to biohazard	3	5	15	4	12
The disposable component of the device shall cost less than 1/10 of typical analyte/test reimbursement	3	4	12	5	15
The disposable component shall interface with the device electronics to support sensor function	3	5	15	5	15
The device shall be RoHs compliant	2	5	10	5	10
The device shall comply with IEC 60601	2	5	10	5	10
The device shall perform analysis at body temperature (37.4 +/- 0.3 C)	2	5	10	5	10
The device leverages open source electronic platforms such as Arduino, Raspberry Pi, Beaglebone Micro controllers	1	5	5	5	5
The device shall operate in any handheld orientation	1	5	5	2	2
Total:	**285**		**279**		**276**

The scores are identical for both designs (279 out of 305), mostly due to their very similar designs. Since the Pugh matrix did not further assist in our final selection process, we looked at other aspects of the design such as feasibility, testing, and timeline.

The peristaltic pump was originally designed to have the pump built onto the cartridge. However, because the pump does not need to come in contact with the fluid sample, the second iteration attached the pump exteriorly from the cartridge to allow for easier installation and maintenance, while the rotor was created to still be attached to the disposable cartridge. The peristaltic pump was more difficult to manufacture than the syringe pump as well due its need for an individually printed micro-rotor on every cartridge as well as the necessity for proper inlet and outlet connections for the silicon tubing that would interface with it. These extra parts not only add difficulty to the manufacturing process, but to the cost per unit. Additionally, peristaltic pumps are not as durable as its syringe pump counterparts.

Although there are many advantages, there are some setbacks to a syringe pump design, such as the difficulty of creating airtight seals on the inlet and outlet and the need to increase the number

of positioning sensors. The syringe pump failure point centers around the seals at the outlet and aspiration inlets, making the device vulnerable to catastrophic systemic failure based on one very integral component. This risk not only endangers our analyzing device and the user to sever biohazard exposure. Nevertheless, the negative pressure of the system allows the device to work against gravity and thus in any hand held orientation. Moreover, the active feedback loop between the microcontroller and the positioning sensors allows for adjustments at any orientation. The disadvantages of the syringe pump design, while large in scope, all mainly fall on the manufacturing side before there is operator interaction with the product. This lead the team to believe that this design, despite the manufacturing difficulties, was the safest for the operator, as well as the most affordable and adaptive.

The syringe pump design is less likely to fail, easier to manufacture, easier to test, and there is equipment availability. After thoroughly discussing each of our potential designs, applying Pugh matrices, and understanding the time feasibility for the project, the final design chosen was the **syringe pump**.

5. Final Design Assembly

5.1 Cartridge Design

The team was tasked with creating a single use disposable cartridge for blood gas analysis. This took the form of a microfluidic cartridge in which reagents are stored over the sensors until a syringe pump draws in the blood through a metal inlet protrusion over the entrance positioning pins and finally over the sensors. The positioning pins are situated at the beginning and end of the sensor pockets which can be seen clearly in *Figure 36*. These pins ensure full coverage of the sensors as well as give insight into the location of the blood sample. The stepper-motor powered syringe pump will pull in exactly 100 μL at a rate of <30μL/sec, positioning the sample over the sensors. Note the volume of blood that can be held by the channel over the sensor is 54.5 μL which is less than the total 100 μL, allowing for the sensors to be cleaned of reagents with the excess blood before analyzing ion content of the sample.

5.1.1 Previous Iteration's Shortcomings

In the previous iteration of the microfluidic cartridge, a reliable air-tight seal was the main concern. The seal of the cartridge had issues when it came to positioning due to the need of negative air pressure to create the differential for exact fluid positioning. In iteration 4**,** the seal was created with the use of a clear epoxy adhesive - however this adhesive seeped into the microfluidic channels relatively often and created areas with minimized channel diameter. This, in turn, caused the alteration of the cartridge's overall fluid flow and created area of turbulence, potentially harming the test sample. With this in mind, it was determined that creating alignment protrusions as well as offsetting the channels in the material, as seen below in *Figure 34*, would be two ways to combat this defect while still using the same adhesive

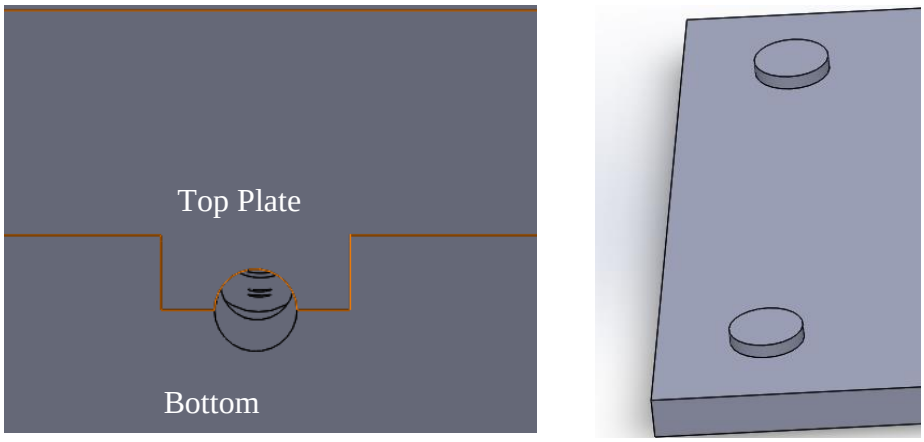

Figure 34: Iteration 4 Channel offset and Alignment Protrusions

5.1.2 Final Cartridge Design

In this final design the team attempted to address all shortcomings of the preceding iterations: reliable seal, incompatible inlet and outlet channel dimensions with syringe tips, distance traveled to sensors, pocketing for hydrogel placement on sensors, sensor placement on top and bottom channels, and

alignment pin placement. With all these suggested modifications the team decided to create a design that had a "tongue in groove" channel offset, alignment bores, and sensor holes on the top and bottom channels. Overall design of top and bottom sections and the assembly of this final design are shown in *Figure 35*, *Figure 36*, and *Figure 37*.

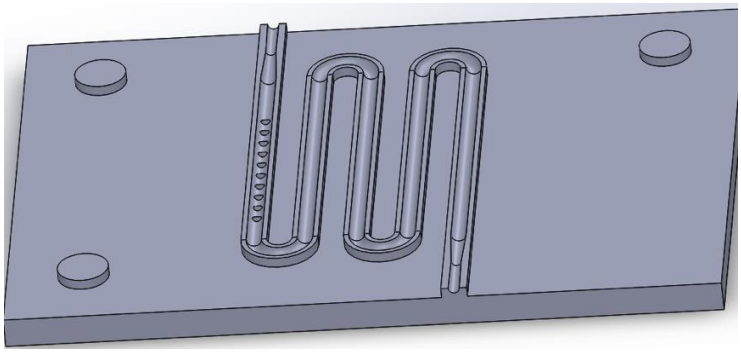

Figure 35: Final Design Cartridge Top Plate

The major advantage of the channels protruding on the top plate is that it allows for the possibility of fitting the channels together before applying adhesive in an attempt to eliminate seepage into the channels. One point to note in this iteration is the inclusion of sensor pocketing on the top plate channels as well as the bottom plate. This is to allow for the potential placement of top and bottom channel sensors, reducing the sample size needed for the same amount of sensors we currently are designing for (seven sensors) or allow for the expansion of tests to be performed through the addition of more sensors.

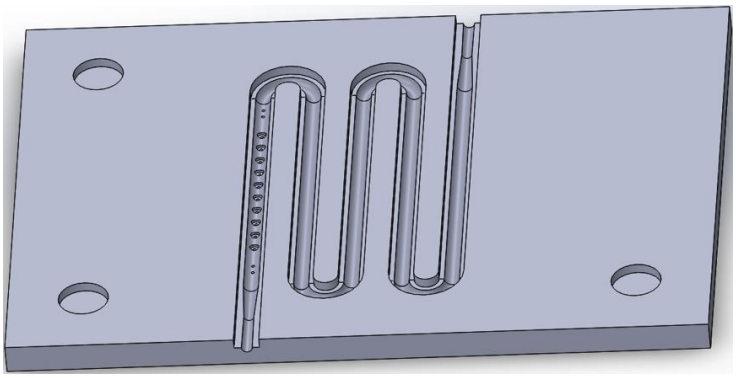

Figure 36: Final Design Cartridge Bottom Plate

Depending on the tolerances determined by the manufacturing process, the bottom plate can be used as a platform for adhesive as well as base for a press-fit seal. If the protruding channels are fit tightly into the depressed groove there can be a liquid tight seal that will allow for the drawing of fluids through the cartridge, and could limit the need for adhesive to the edges of the plates and away from the channels. This fit can be seen in *Figure* 37. Note that only the bottom plate contains the positioning pins.

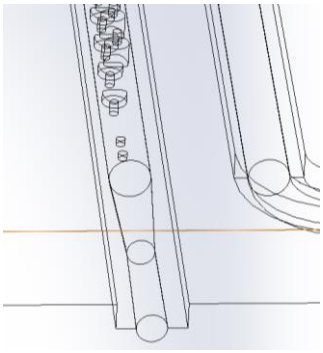

Figure 37: Final Design Cartridge Top and Bottom Plate Assembled (Wire View)

5.1.3 Overall Specifications of Final System Design

Table 6: Summary of Final Device Design

Specification	**Value or Parameter**
Total Volume of Channels	344.8 μL
Volume to Cover Sensors	54.5 μL
Volume of Inlet Pin	5.54 μL (if pin is 10mm long)
Maximum Flow Rate (MFR)	15 μL/sec
Max Pressure for Epoxy Sealant	3200 psi
Rough Cost Estimates (See Calculations)	
PMMA Material Cost (Injection Molding)	$0.00732 per cartridge
PMMA Material Cost (Milling)	$0.31 per cartridge

5.2 Syringe Pump Design

In order to create a functioning syringe pump, three major constraints needed to be taken into account: design footprint, rotational to linear motion conversion, and torque needed for this conversion. With these constraints outlined, the team moved forward and prototyped two different designs. The first design utilized a threaded plate and a machined screw as a conversion of rotational motion. The second design was based on a rack-and-pinion system with additional gearing to increase or decrease the ratio of rotational to linear motion.

5.2.1 1st Prototype: Moving Plate Design

This design utilized a threaded plate fixed to two non-threaded plates sandwiching the end of the syringe. The overall finished product is depicted in *Figure 38*.

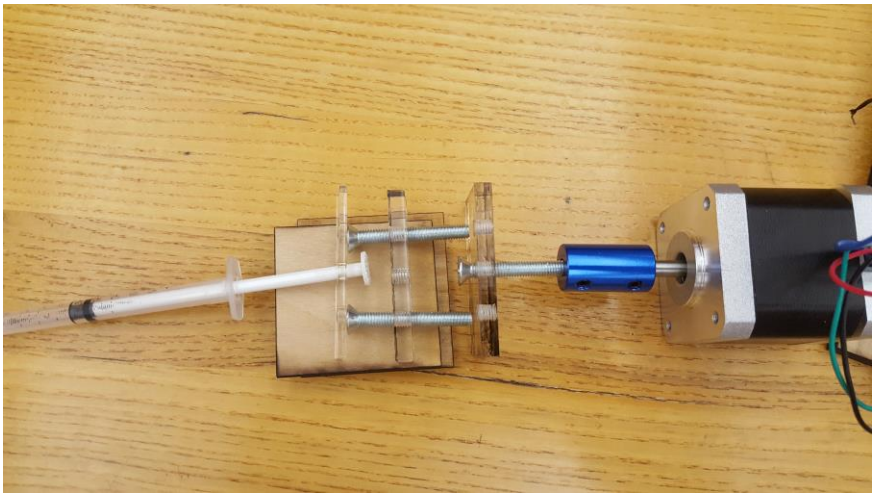

Figure 38: Overall Setup of 1st Prototype

Strengths of this design include: minimal design footprint, rotational to linear movement ratio is very high (meaning there can be a lot of rotational movement to minimal linear movement), and the setup is very cost effective. One pitfall to this design is the need for consistent torque and the possibility of the screw slipping off the plate.

Manufacturing of this prototype was done with the laser cutter with ¼ inch acrylic and with a manual tap of ¼ inch in diameter. These parts were then combined with 4 10-20 x 1 ½ inch screws. The AutoCAD template used on the laser cutter is shown in

Figure 39 with dimensions in inches.

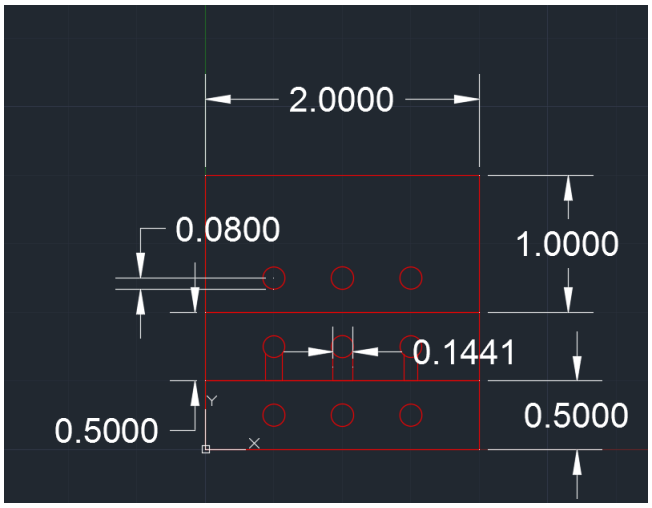

Figure 39: Stepper Plates AutoCAD Drawing, Dimensions in Inches

The above shown plates have three different purposes in the syringe pump use. The top plate is used as the master motion plate, and is the only plate being physically moved directly through the rotation of the screw through its center hole. The bottom plate is the secondary plate, these plates are fixed to the master plate using screws in the outer two holes and translate this linear motion to the

plunger of the syringe. The middle plate is the attachment plate and it sandwiches the syringe plunger, ensuring that the syringe can both pull fluid in as well as be reset to its original position for the next run.

The outcome of this first iteration of the prototype was slipping in the plates resulting in inconsistent linear movement. In order to address these issues the team manufactured a base holder out of wood and modified the plate design to be made out of metal. Seen in *Figure 40*, *Figure 41*, and *Figure 42*.

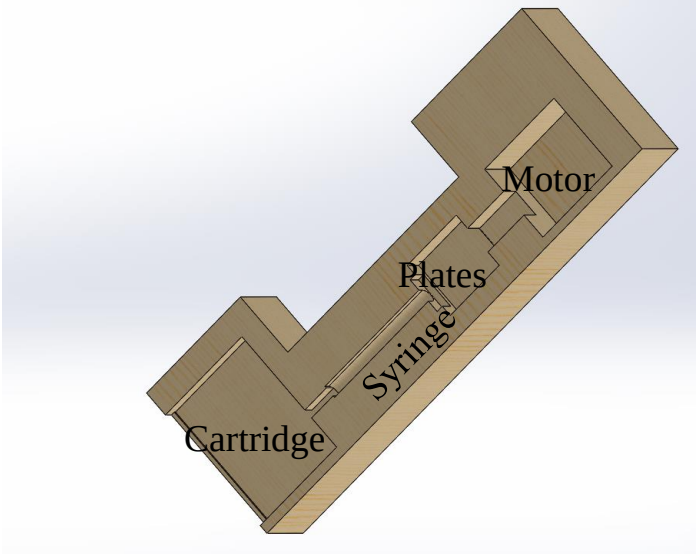

Figure 40: Base Holder SOLIDWORKS 3D Model

This base model allows for the interfacing of all previously designed components: the syringe pump (comprised of the syringe, moving plates, and motor) and the microfluidic cartridge. As shown above the cartridge is inserted at the bottom of the device and interfaces with the syringe through a blunt syringe tip previously inserted at the top of the cartridge. Below are the dimensioned drawings for both the base housing and the plates for linear motion.

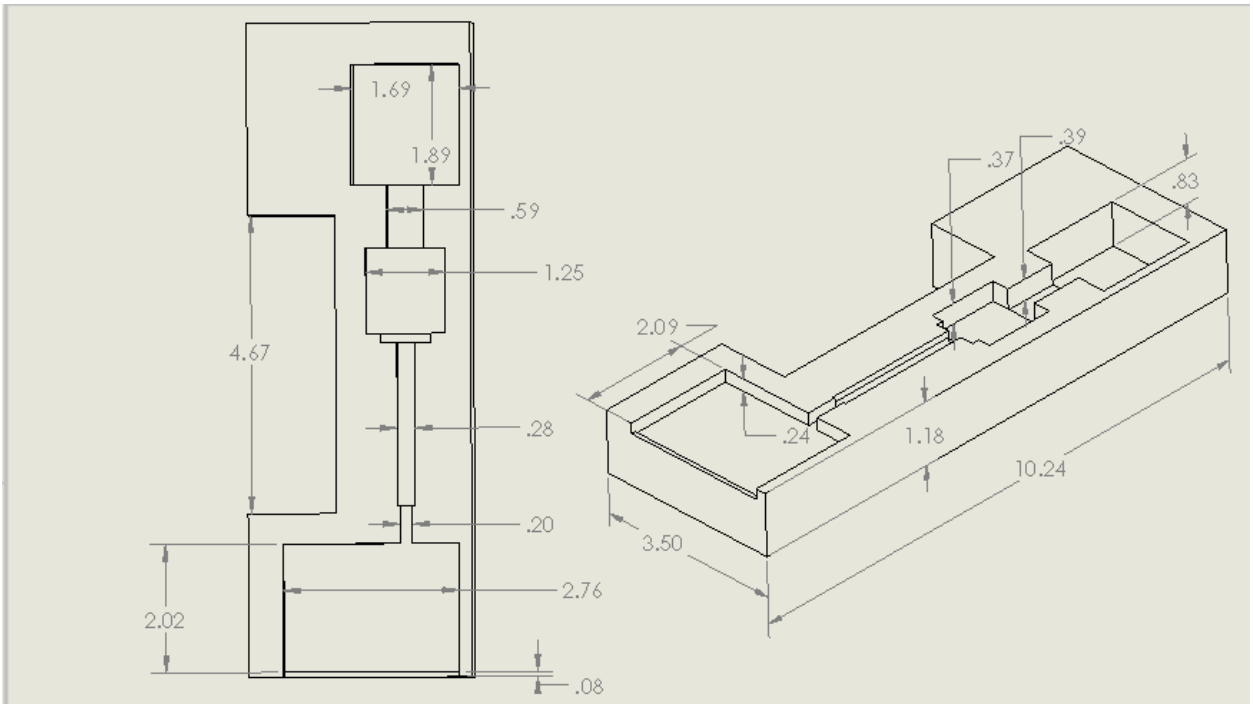

Figure 41: Base Holder SOLIDWORKS Dimensioned Drawing (in)

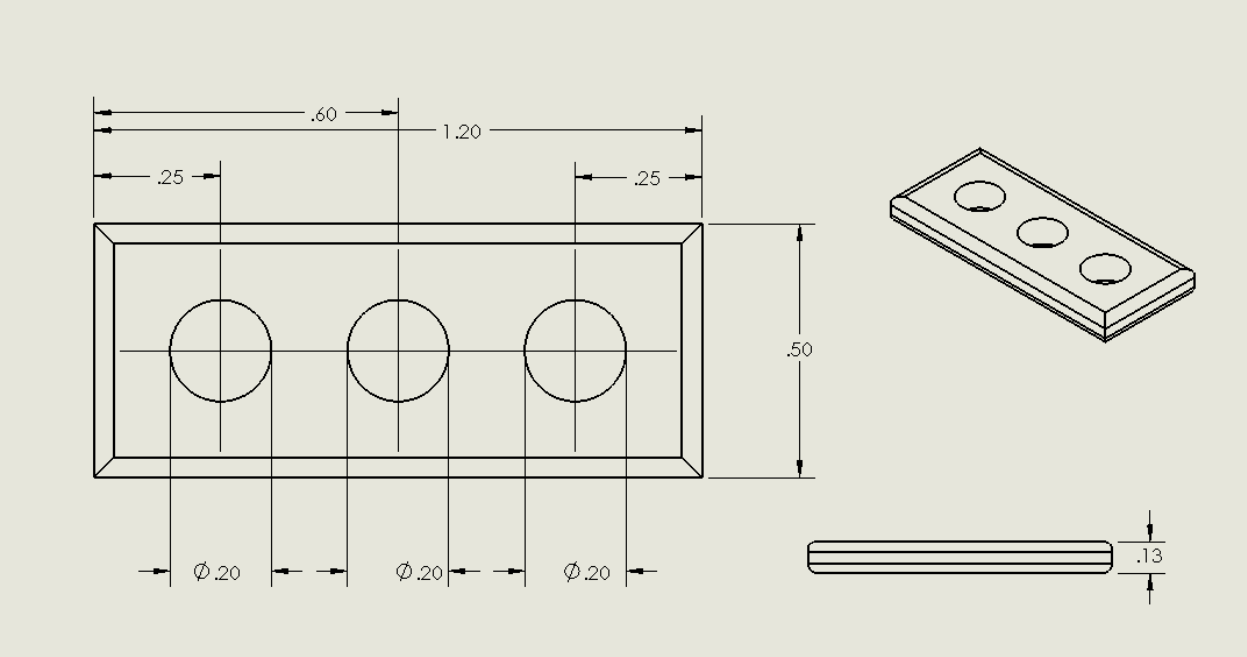

Figure 42: Linear Motion Plates SOLIDWORKS Dimensioned Drawing (in)

One thing to note is the lack of 3 distinct plates for the latest iteration of the moving plate design. This is due to uniformity needed for the base holder as well as that the team found the other plates to be superfluous.

5.2.2 2nd Prototype: Geared Design

This design utilized the rack and pinion conversion of rotational motion to linear movement depicted in more detail in *Figure 43*.

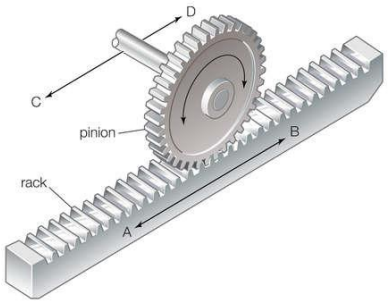

Figure 43: Rack and Pinion, Rotational to Linear Motion

In order to allow for the alignment of the stepper motor to ensure the movement of the rack a baseplate was designed as well for optimization of setup. The overall setup of the 2nd prototype is shown below.

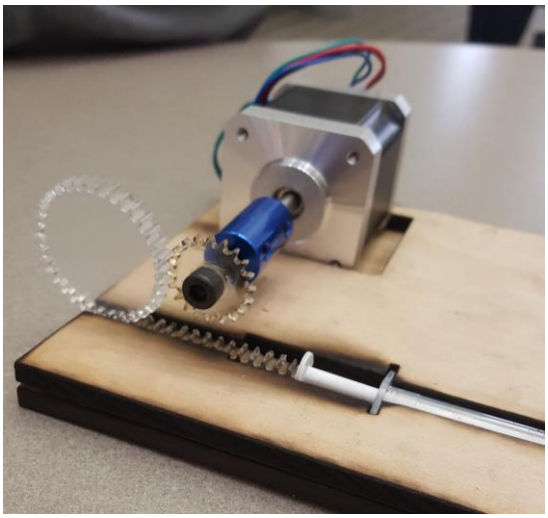

Figure 44: Overall Setup of 2nd Prototype

Strengths of this design include a high accuracy with rotational to linear conversion, a low level of necessary torque, and a high precision with movement. Weaknesses of this design are: a large design footprint, a high level of assembly complexity, and the possibility of gears not meshing.

This design required various calculations in order to generate the correct gearing ratio to move the rack at a slow enough rate. One major tool used in this process was the Gear Generator app from geargenerator.com[70][69][69][70][70][69][69][69][69][69][69][69][69][69][69][66][66][66][66][70][69][69][68][67][67][67][66][67][67][68][68][68]. This app allows the user to input the number of teeth and the input RPM in order to find the correct ratio of gears. The overall setup of the final decided upon gearing is shown below in *Figure 45*.

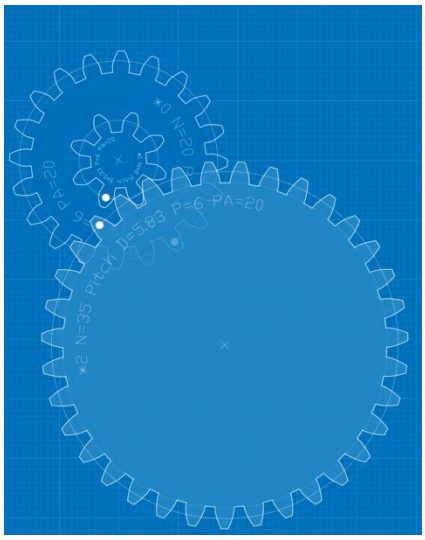

Figure 45: Gearing Setup for Stepping down RPM

In *Figure 46* below details the RPM change that each gear experiences in the setup shown above.

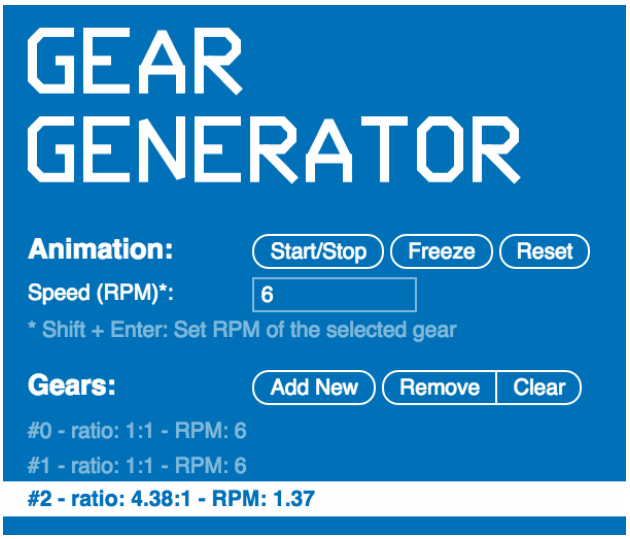

Figure 46: Calculated RPM Step Down

Manufacturing of this prototype was done with the VLS 4.6 laser cutter with ¼ inch acrylic for the gearing and rack, and with ¼ hard wood for the baseplate. The AutoCAD templates used on the laser cutter are shown below in *Figure 47* through *Figure 51*.

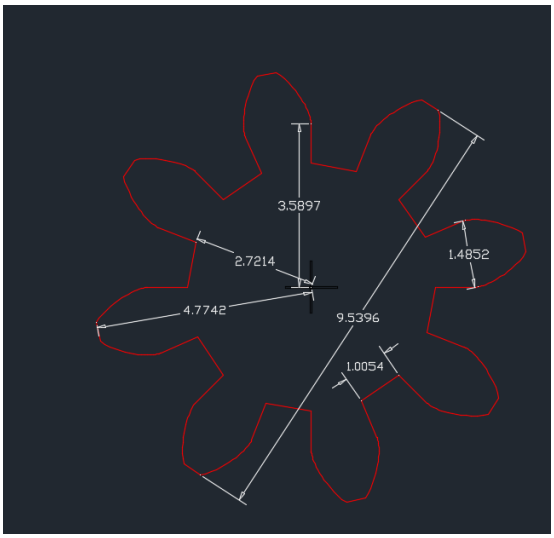

Figure 47: 8 Toothed Gear AutoCAD Drawing, Dimensions in mm

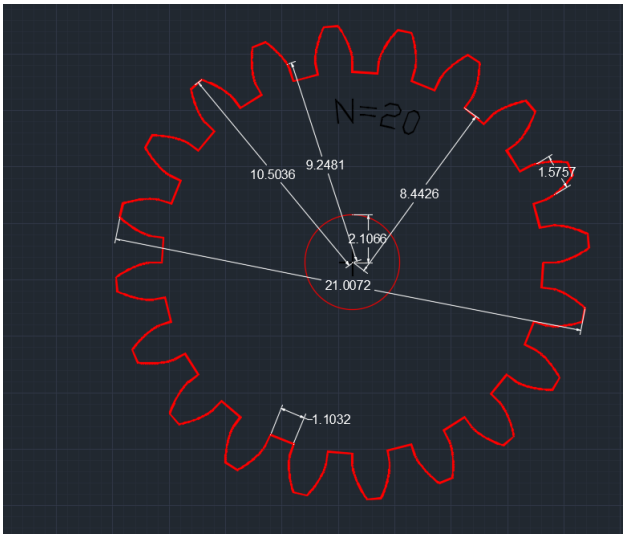

Figure 48: 20 Toothed Gear AutoCAD Drawing, Dimensions in mm

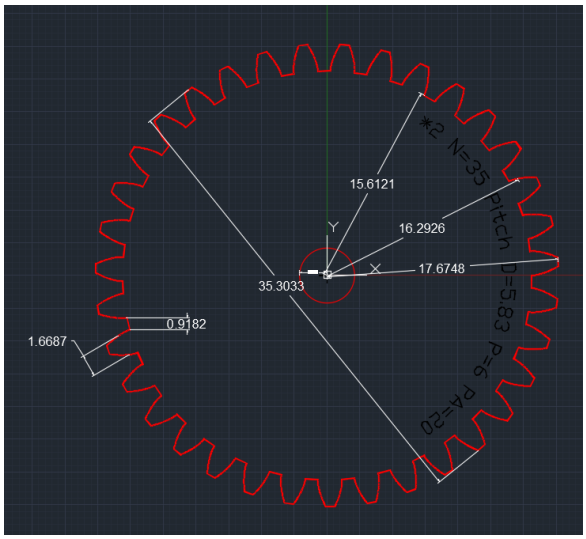

Figure 49: 35 Toothed Gear AutoCAD Drawing, Dimensions in mm

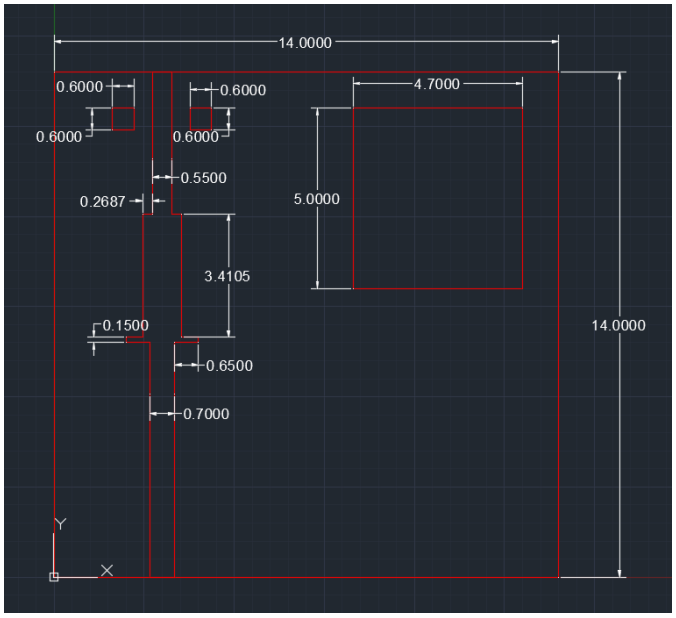

Figure 50: Base Plate for Holding Syringe, Stepper Motor, Rack and Gears, Dimensions in cm

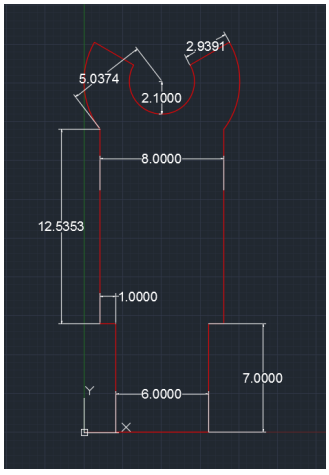

Figure 51: Gear Holder AutoCAD Drawing, Dimensions in mm

5.2.3 Final Syringe Pump Design

Throughout the process of fabricating and implementing the two prototypes, we reached the final decision to use the moving plate design due to its simplicity, ease of fabrication, and versatility when it came to reiterations and refining of the design by removing the major reliance of alignment the gear design had. In order to further refine the design, the team moved forward with manufacturing their own custom plates out of both aluminum and zinc plated steel to test the rigidity and usability. The team also created a custom housing in which the syringe pump could operate, milled out of pine, with a slot for the insertion of the cartridge as well as a top cover with holes for LED's and the interactive LCD screen. This final product is shown below.

5.3 Calculations

$$\pi * r^2 * h \quad (11)$$

$$surface\ area\ rectangle = length * width \quad (12)$$

$$volume\ of\ a\ rectangular\ prism = length * width * thickness \quad (13)$$

$$total\ volume = Total\ weight * Density \quad (14)$$

$$Material\ Cost\ per\ Cartridge = \frac{Total\ Material\ Cost}{Number\ of\ Chip\ Fabricated} \quad (15)$$

Total Volume channels

$$Total\ Volume\ by\ Length = \sum Straight\ Channels\ (cylinders) + \sum Curves\ (Cylinders) \quad (16)$$

$$Total\ Volume = 5(\pi *.76^2 * 30) + 4(\pi *.76^2 * 10) = 272.2\ mL + 72.6mL$$
$$Total\ Volume = 344.8\ mL$$

Volume Inlet Pin

$$Total\ Volumeof\ Inlet\ Pin = Volume\ Pin = \pi * r^2 * h = \pi * (0.42mm)^2 * 10mm = 5.54\ mL \quad (17)$$

Total Cartridge Material Needed

$$Material\ Needed\ Volume = Volume\ Top\ Plate(rec.prisim) + Volume\ Bottom\ Plate(rec\ prism) \quad (18)$$
$$= 3mm * 51mm * 70mm + 4mm * 51mm * 70mm = 10710mm^3$$
$$Material\ Needed\ Volume = 10.71cm^3$$
$$Material\ Needed\ Surface\ Area = 2(51mm * 70mm) = 7140mm^2 = 0.00714m^2 = 11.07in^2$$

Total Material Cost (Injection Molding)

Cost = $800 average for 1 metric ton
Density = 1.17g/cm^3

$$1\ metric\ ton = 1{,}000{,}000\ grams\ \therefore\ Total\ Volume = 1{,}000{,}000 * \frac{1.17g}{cm^3} \quad (19)$$
$$= 1{,}170{,}000cm^3$$
$$1\ cartridge\ (top\ and\ bottom) = 10.71cm^3$$

$$\frac{1{,}170{,}000cm^3}{10.71cm^3} = 109{,}243.46 = 109{,}243\ chips$$

$$Total\ Material\ Cost = \frac{\$800}{109{,}243} = \$0.00732\ per\ cartridge$$

Total Material Cost (Milling)

Sheet of 0.177in (4.5mm) x 51" x 100" (cheapest sheet option when you buy sheets in bulk 50+)
Cost = $140.53
Surface Area per cartridge = 11.07in^2
Total Surface area sheet = 5,100 in^2

$$Total\ Material\ Cost = \$140.53 \div round\left(\frac{5{,}100}{11.07}\right) = \$0.31\ per\ cartridge \quad (20)$$

5.4 Fabrication

5.4.1 Cartridge Fabrication

With our design now chosen, we explored potential materials and fabrication methods available to us through IL and also WPI. We saw that our most cost effective and fastest method for initial iterations was to have the machine shop at IL micro-mill our plate designs into clear casted acrylic sheets. Our initial 3D CAD models created in SOLIDWORKS were saved as SLDPRT files to be loaded into the machine shop's software.

In order to create a water tight seal between the two plates, several bonding methods were investigated. At first, our team pursued a clamping design using aluminum plates and screws, but found quickly that due to the surface bonding properties this was not a viable option. We then progressed to using a water tight epoxy resin that would provide a water tight seal around the channels. However, one of the major issues with this method was that application technique varied greatly with the fabricator. The epoxy often times hardened on contact with the acrylic before the top plate could be put on, and also sometimes bled into the engravings, occluding the channels. However, several cartridges were successfully made with this method and thus were tested with.

5.4.2 Syringe Pump: Physical Design

5.4.2.1 Overall Design Considerations

In order to create a functioning syringe pump three major constraints needed to be taken into account: the design footprint, the rotational to linear motion conversion, and the torque needed for this conversion. With these constraints, the team moved forward and prototyped two different designs. The first design utilized a threaded plate and a machined screw as a conversion of rotational motion. The second design was based on a rack-and-pinion system with additional gearing to increase or decrease the ratio of rotational to linear motion.

5.4.2.2 1st Prototype: Moving Plate Design

This design utilized a threaded plate fixed to two non-threaded plates to hold the end of the syringe. The overall finished product is depicted in *Figure 52*.

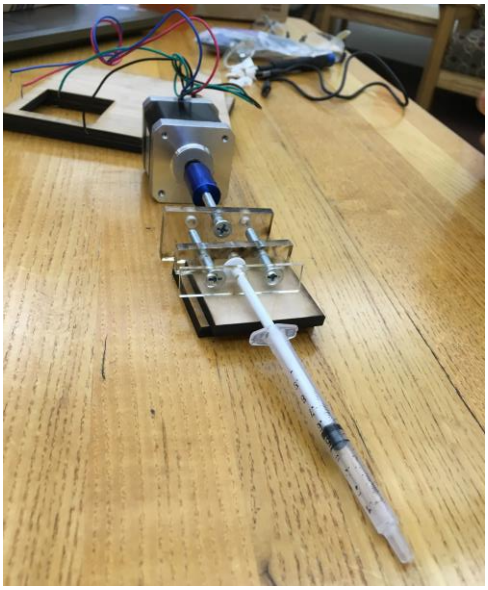

Figure 52: Overall Setup of 1st Prototype

Strengths of this design include: minimal design footprint, rotational to linear movement ratio is very high (meaning there can be a lot of rotational movement to minimal linear movement), and the setup is very cheap. One pitfall to this design is the need for consistent torque and the possibility of slipping of the screw in the plate.

Manufacturing of this prototype was done with the laser cutter with ¼ inch acrylic and with a manual tap of ¼ inch in diameter. These parts were then combined with 4 10-20 x 1 ½ inch screws. The AutoCAD template used on the laser cutter is shown below with dimensions in inches.

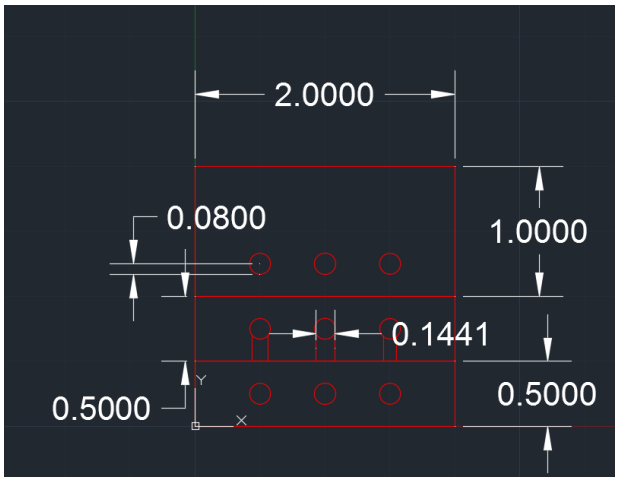

Figure 53: Stepper Plates AutoCAD Drawing, Dimensions in Inches

5.4.3 2nd Prototype: Geared Design

This design utilized the rack-and-pinion conversion of rotational motion to linear movement depicted in more detail in *Figure 54*.

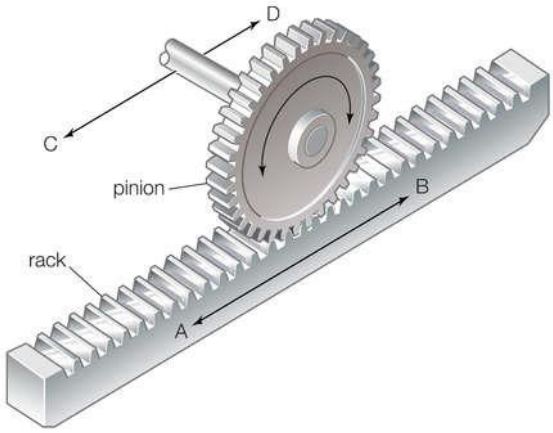

Figure 54: Rack and Pinion, Rotational to Linear Motion

In order to allow for the alignment of the stepper motor to ensure the movement of the rack a baseplate was designed as well for optimization of the setup. The overall setup of the 2nd prototype is shown in *Figure 55*.

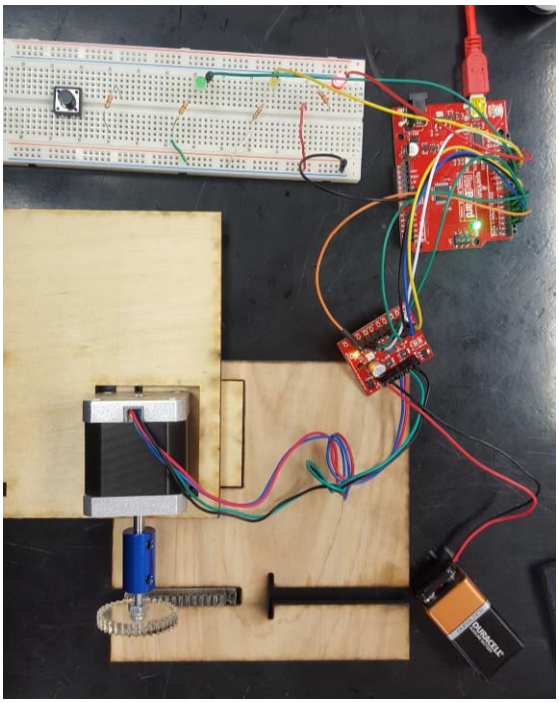

Figure 55: Overall Setup of 2nd Prototype

Strengths of this design include: high accuracy with conversion from rotational to linear, very little torque needed, and high precision with movement of the syringe. Weaknesses of this design are: a large design footprint, complexity with assembly, and the possibility of gears not meshing.

This design required a lot of calculations in order to generate the correct gearing ratio to move the rack at a slow enough rate. One major tool used in this process was the Gear Generator app from geargenerator.com. This app allows the user to input the number of teeth and the input RPM in order to find the correct ratio of gears. The overall setup of the gearing is shown in *Figure 56*.

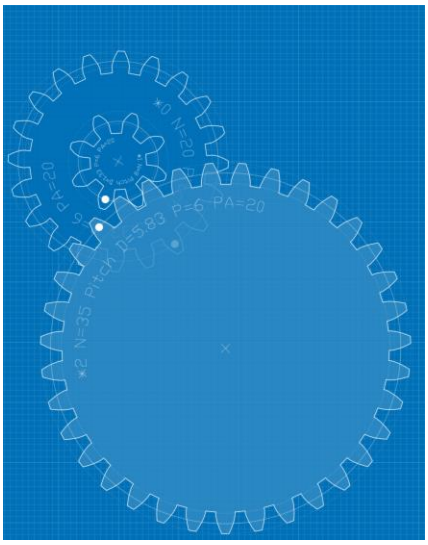

Figure 56: Gearing Setup for Stepping down RPM

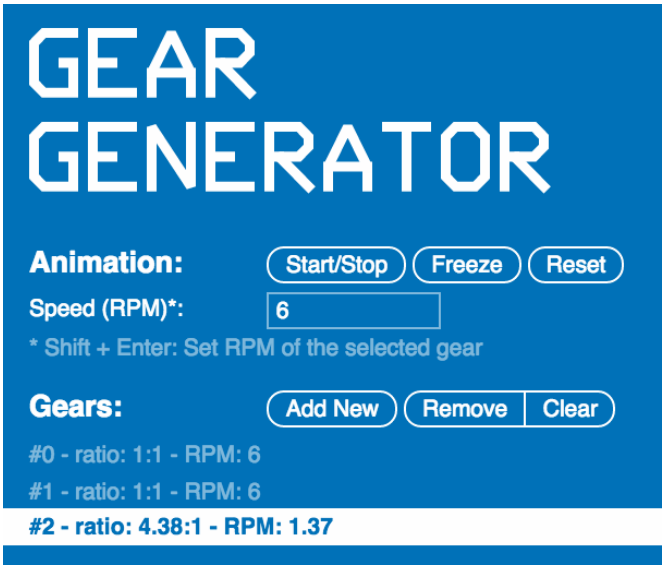

Figure 57: Calculated RPM Step Down

Figure 56 details the RPM change that each gear experiences in the setup shown. Manufacturing of this prototype was done with the laser cutter with ¼ inch acrylic for the gearing and rack, and

with ¼ hard wood for the baseplate. The AutoCAD templates used on the laser cutter are shown in *Figure 58- Figure 62*.

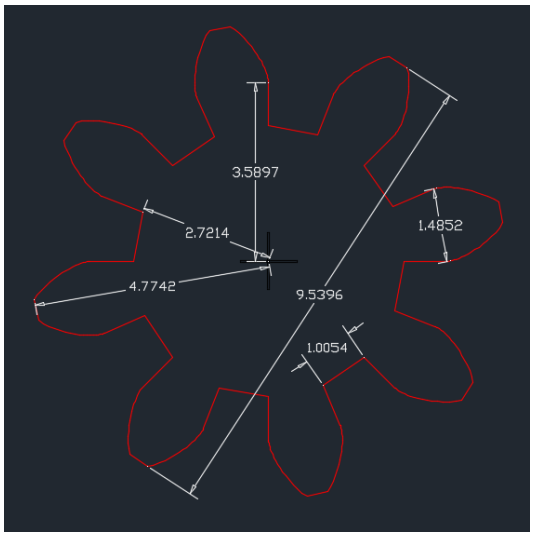

Figure 58: 8 Toothed Gear AutoCAD Drawing, Dimensions in mm

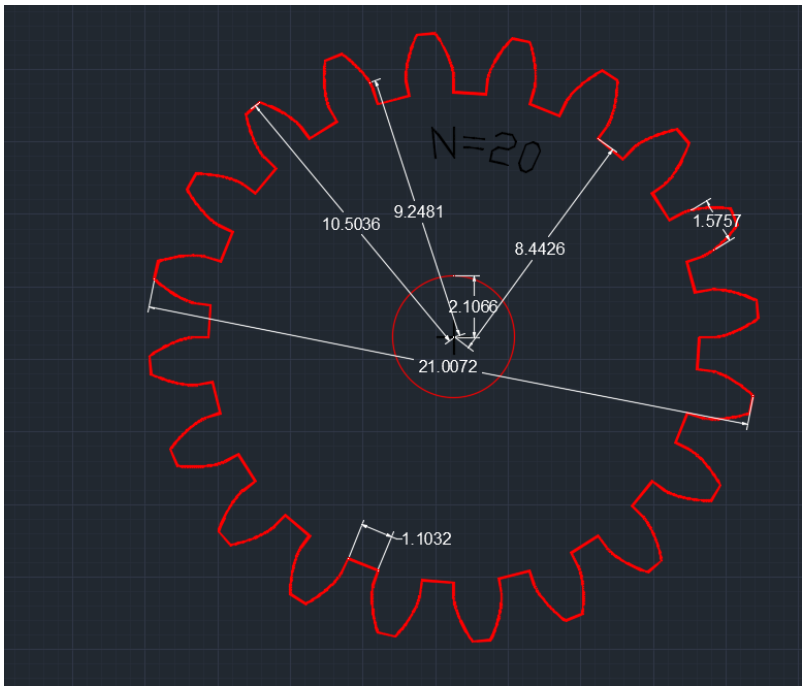

Figure 59: 20 Toothed Gear AutoCAD Drawing, Dimensions in mm

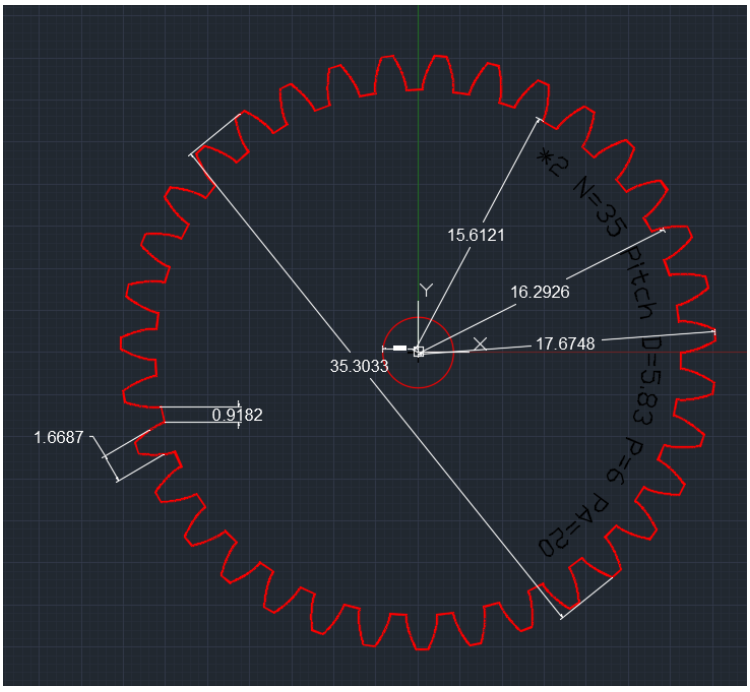

Figure 60: 35 Toothed Gear AutoCAD Drawing, Dimensions in mm

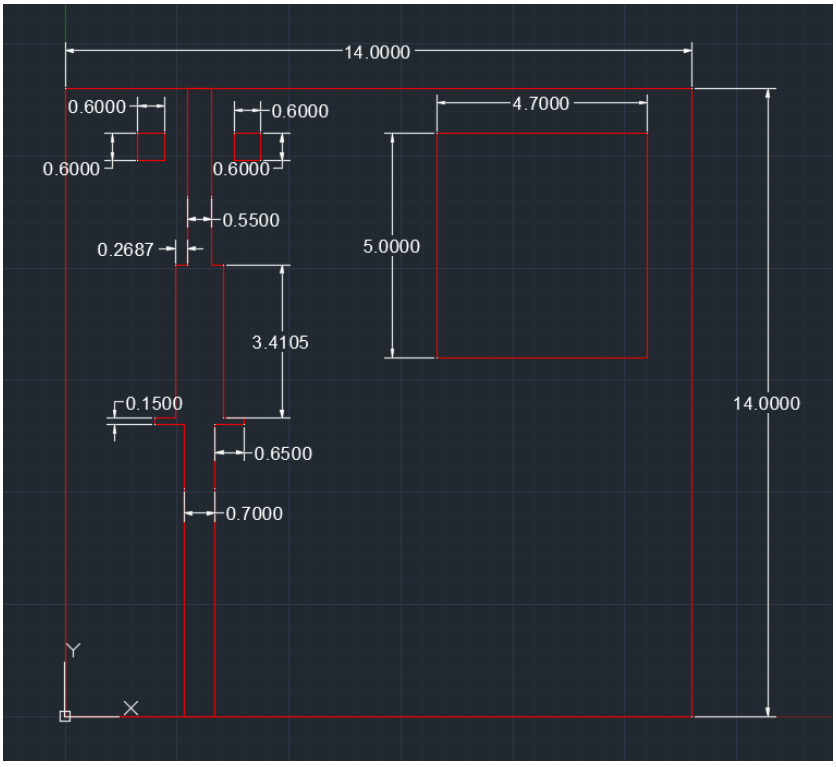

Figure 61: Base Plate for Holding Syringe, Stepper Motor, Rack and Gears, Dimensions in cm

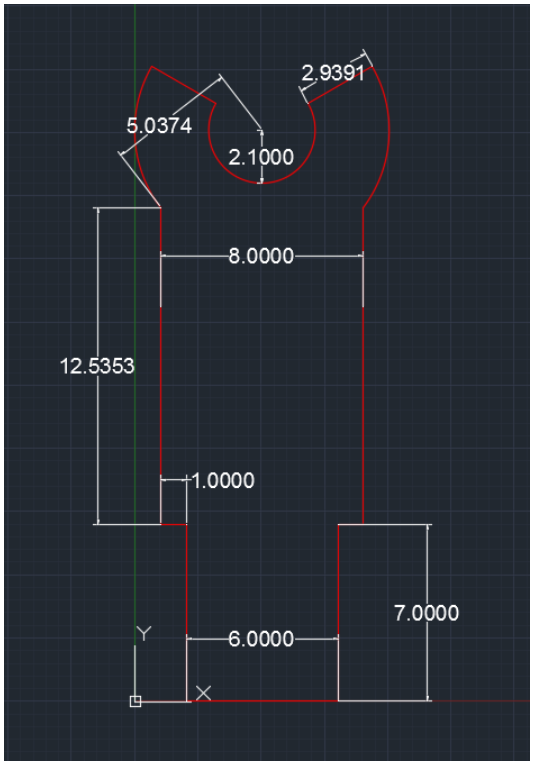

Figure 62: Gear Holder AutoCAD Drawing, Dimensions in mm

5.1.2.4 Conclusion

After the completion and application of both prototypes, it was clear that considerable refinements were needed in the designs. While both had their strengths and weaknesses, the final decision for best rotational to linear movement translation was the deciding factor in addition to several other characteristics. If the design footprint was the main constraint, then the moving plate prototype should have been the one to be further refined. If the accuracy of movement is more important, then the geared design should have been refined. The team eventually selected the **plate design** due to its high linear to rotational resolution as well as it lower design foot print.

5.4.3 Syringe Pump: Controls Design

The stepper motor runs at a rate of 400 steps per revolution at full step resolution with a step angle of 0.9° [71]. It has a holding torque of 48N·cm. Its fastest speed is 1.12 rev/sec while its slowest speed is 0.068 rev/sec. These translated to volumetric flow rates of 960 µL/min and 58 µL /min respectively. The datasheet for this stepper motor can be found in
. The fastest speed has a microstep resolution of Full Steps while the slowest uses Sixteenth Steps.

To control the stepper motor, we used a SparkFun RedBoard while programming with Arduino [72]. The Big Easy Driver is a stepper motor driver board that was used to connect the bi-polar stepper motor to the RedBoard [73]. The first step was to connect the correct, color-coded wires from the stepper motor to the driver board. These connected the motor to Coil A+, A-, B+ and B- to power and control the stepper motor. We also connected the jumper wires for a 9V battery pack on the same side of the driver for the motor. Note, the battery does not get plugged in until the stepper motor is ready to run. The next step was to connect the RedBoard microcontroller to the driver board. The

following pins needed to be connected: STEP, DIR, MS1, MS2, MS3, ENABLE, and GND. These were connected to pins D2-D7 as well as ground. The final circuit setup should is shown in *Figure 63*.

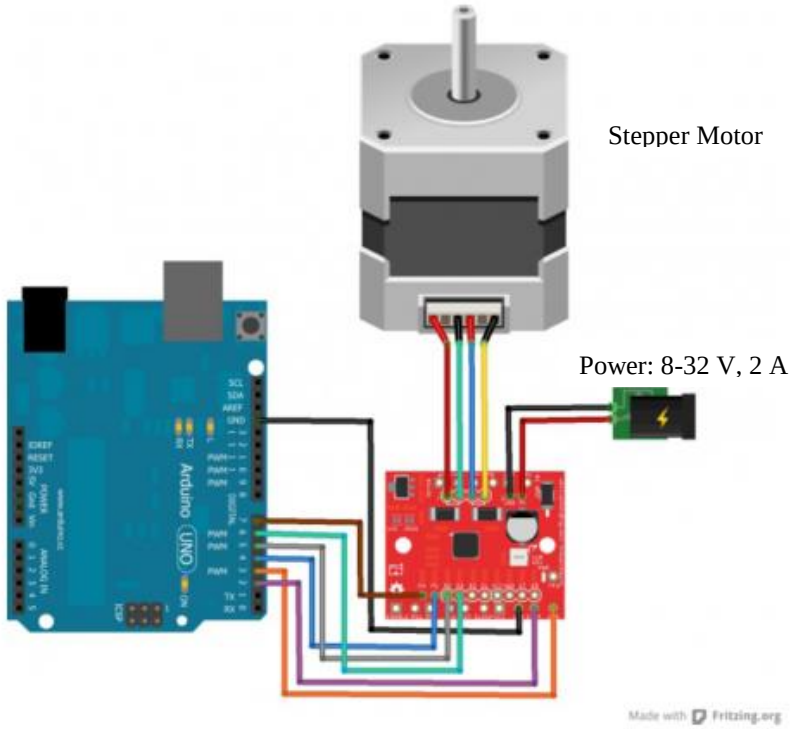

Figure 63: Final Circuit Set Up

5.5 Arduino Code Programming

The code used for the stepper mode can be found in *Appendix C: Stepper Motor Code*. We used an open source Arduino program that was packaged with the stepper motor. It gave us the basics of declaring pin functions, setting up the serial input and output, and the initial code for controlling the stepper motor which include following truth tables. *Table 7* shows the general pin control and *Table 8* displays how to enable different microstep resolutions.

Table 7: Pins Truth Table

Pins Truth Table		
Pin	**Mode**	**Control**
STEP	Low	Stepper motor is off
STEP	High	Stepper motor takes a step
DIR	Low	Moves stepper motor in forward direction
DIR	High	Moves stepper motor in reverse direction
ENABLE	Low	Disables FETs
ENABLE	High	Enables FETs, allowing motor control

Table 8: Microstep Resolution Truth Table

Microstep Resolution Truth Table				
MS1	**MS2**	**MS3**	**Microstep Resolution**	**Excitation Mode**
L	L	L	Full Step	2 Phase
H	L	L	Half Step	1-2 Phase
L	H	L	Quarter Step	W1-2 Phase
H	H	L	Eighth Step	2W1-2 Phase
H	H	H	Sixteenth Step	4W1-2 Phase

We connected three LEDs on a bread board to pins D12, D11, and D10, representing red LED1, yellow LED2, and green LED3 respectively. LED1 lights up to show the system is ready to begin and blinks when the stepper motor steps. LED2 blinks to represent sensor reading status. LED3 lights up when the reading is complete and prompts the user to reset the system. *Figure 64* shows the circuit diagram used to connect the 3 LEDS in parallel to the Arduino UNO board.

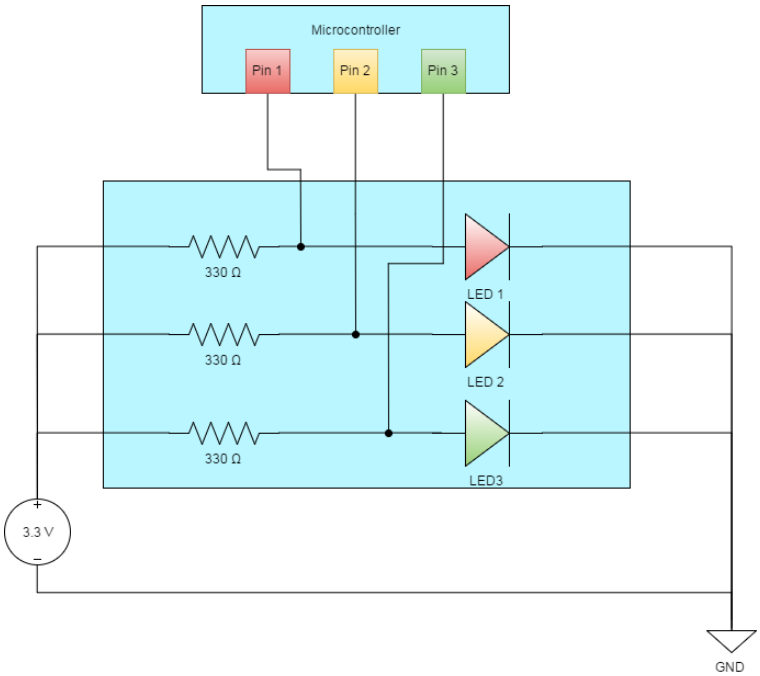

Figure 64: Circuit Diagram Used to Connect LEDs to Arduino UNO

We programmed the board to run in Sixteenth microstep resolution. The serial motor begins by lighting LED1 and prompting the user for three input options: "1" is to pull the stepper motor in the forwards direction at 1/16 microsteps, "2" pulls the stepper motor in the reverse direction, "3" blinks LED2, a place holder option until the sensors are introduced to the system. After reading the user input, the system will either run the stepper motor forwards or reverse or blink LED2. The stepper motor is controlled to those options using the truth tables above and performing **DigitalWrite(Pin, Mode).** A for loop steps the motor **x** times, changing the STEP pin high and low with each loop. The LED also turns on and off every couple of loops, using modulo 50 and 100. This means that at a faster microstep resolution, the LED will blink faster as well.

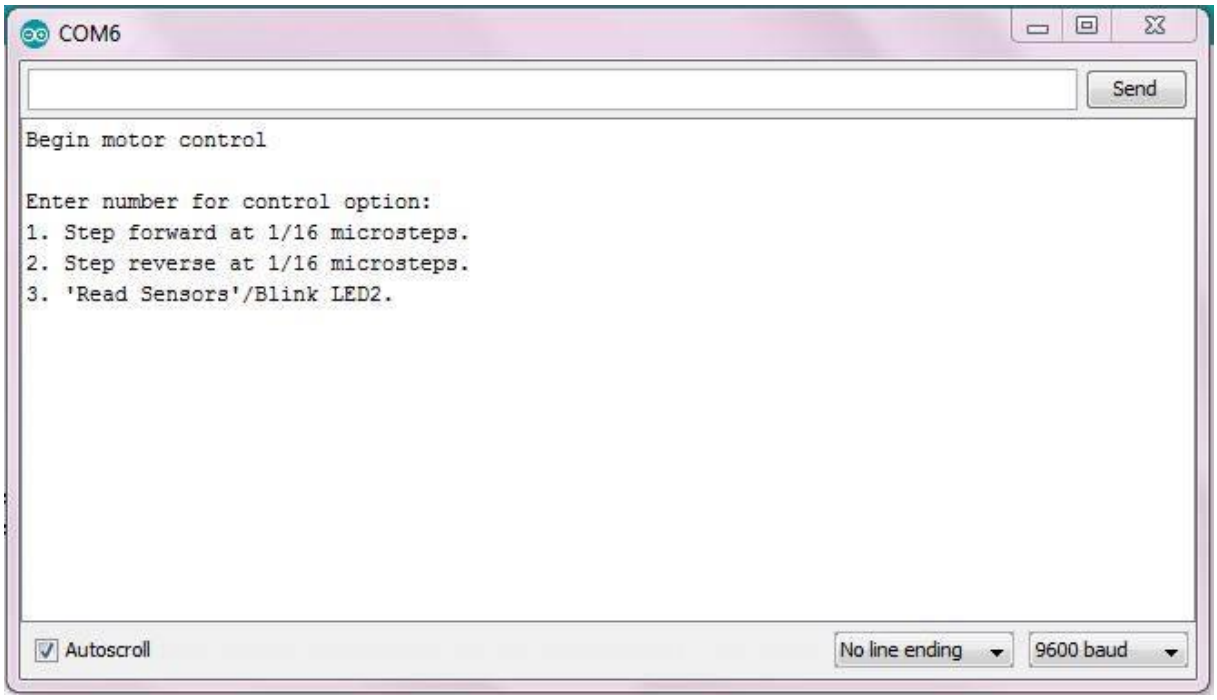

Figure 65: GUI Interface of Stepper Motor Code

After running one of those options, the serial motor prompts the user to input another option or input "x" to reset the system. Currently, LED3 will only light up after LED2 blinks, representing that the system is only complete after the sensor readings is complete.

The different pin modes allows for more control over the stepper motor. At any time, the code could be changed to different microstep resolutions to change the speed and volumetric flow of the system, pulling in fluid at different rates. *Figure 65* shows the GUI interface used to control the stepper motor.

6. Design Verification

The team devised a set of 8 testing protocols in order to evaluate the effectiveness and quality of our device. The full protocols can be found in *Appendix A*: Testing Protocols . The subsequent sections will provide a short summary of each test procedure.

6.1 Protocols

6.1.1 Blood Substitute Testing (Viscosity and Grain Size)

Due to the limited availability of human blood for testing, the need for a sample substitute was of great importance to this project. With this in mind, it was proposed that a combination mixture of rice starch and water be used, due to the similarities in size and delicacy of the rice granules to red blood cells. Thus various ratios of rice starch to water were prepared and tested with a Brookfield DV2T viscometer rotating at 50 RPM using spindle number 18. Viscosity was measured as the average of the readings over 120 seconds of spindle rotation. A projection line using the measured rice starch mass to distilled water ratio was also created in order to estimate the correct ratio needed to reach a viscosity of 3.1 cP.

Additionally, the granule size of the rice starch was also characterized. Using a 0.05 g/mL solution of rice starch to water, 10 µL of the sample was placed on a hemocytometer and observed under a microscope. ZEN imaging software was used to measure grain diameter for a quadrant, as shown in *Figure 66*.

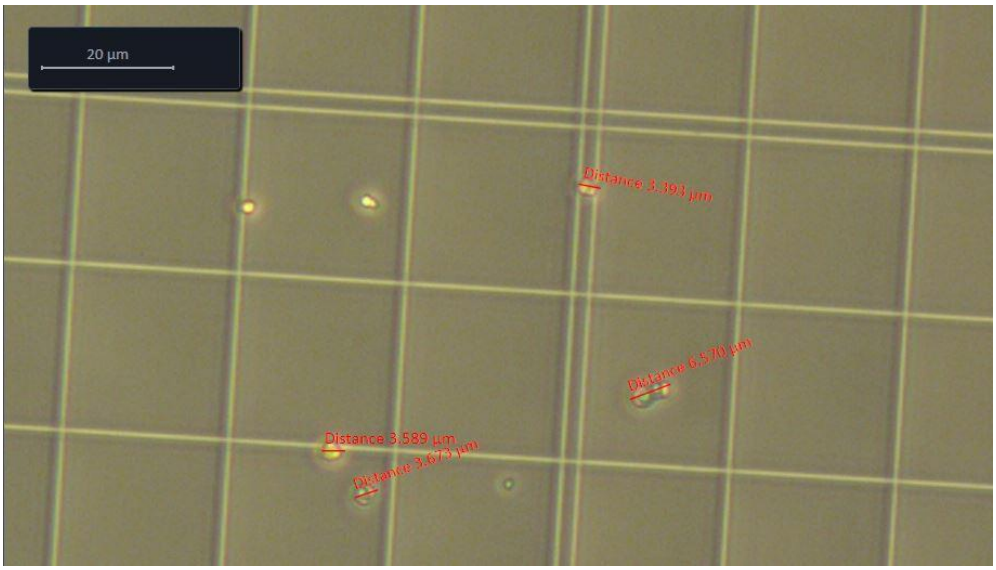

Figure 66: Measuring rice grain diameter using ZEN imaging software

6.1.2 Cartridge Seal Testing

A sealing test was performed in order to ensure that a minimum of 60 µL of sample is placed over the sensors while also preventing biohazard exposure to the user. *Figure 67* summarizes the procedure: we took an assembled cartridge (i.e. the two plates have been adhered together using epoxy) and imaged the sections of channel containing sensors and surrounding area using microscope equipped with a camera. Next, 100 µL of test fluid was positioned over the channels with sensors using a 200 µL pipette. We then allowed the cartridge to sit for 5 minutes. The cartridge was then again placed under

a microscope and images of the sensor areas were once again taken and saved. The area directly adjacent to the channel edge was characterized using the ZEN imaging software to discern the RGB (Red, Green, and Blue) values before and after fluid was introduced into the channel. RGB is a numerical way that computers characterize colors based on how much red, green, and blue is in the image. The difference in RGB values greater than 30, a value experimentally determined that denoted leakage, were noted.

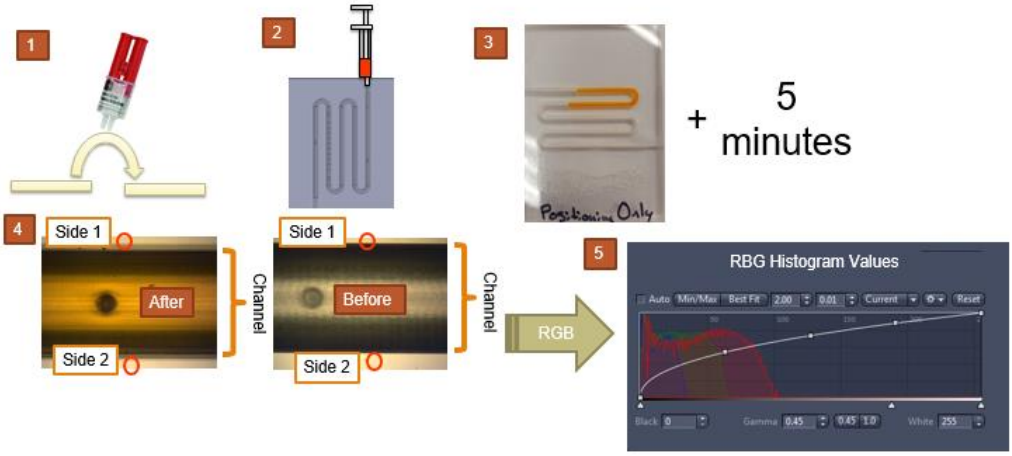

Figure 67: Summary of Sealing Test Procedure

6.1.3 Vertical and Horizontal Orientation Testing

One major concern with our device design was potential leakage of the sample from the inlet or outlet ports after withdrawing the disposable cartridge from the handheld device. With this in mind, additional lengths and curves in the channel track were created. In order to verify the cartridge did not allow fluid to back flow out through either opening. To do so, cartridges containing 100 µL of fluid were flipped along the x and y axis by 0°, 45°, 65°, and 90° along both the x (shown in *Figure 68*) and y axis, then checked for any leakage out of the inlet and outlet.

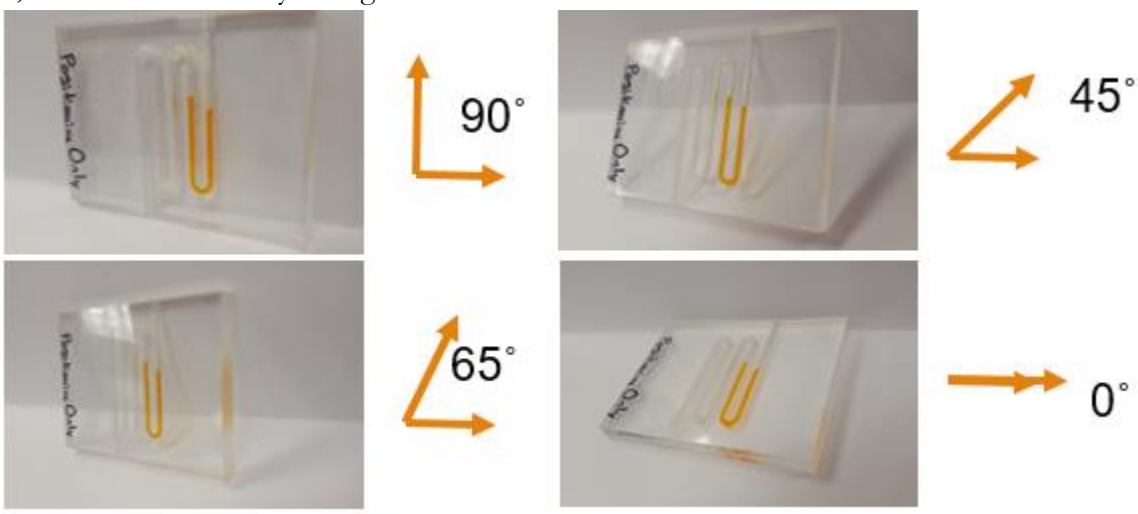

Figure 68: Rotation along Different Angles along the X Axis to Check for Fluid Containment

6.1.4 Temperature Control

Additionally, keeping the test at 37°C would allow for best reading of the sample by maintaining it at body temperature. With this being said, we can test to see if the sample passed through does reach 37°C when placed over the sensors by creating a cartridge with the appropriate channel distance. The testing fluid was warmed to 37°C prior to testing and was then injected into the cartridge using a 200 µL pipette. 66 gauge thermocouples, made by Trevor Noah of the Fire Protection Laboratories at WPI, measured the temperature at the inlet, outlet, and sensor areas of the microfluidic channels over the course of 300 seconds. A heating plate was used as the main temperature control and was set to 35°C, 45°C, and 75°C .This would allow for characterization of the cartridge fluid temperature over a certain distance related to not only placement but also heating element temperature wise.

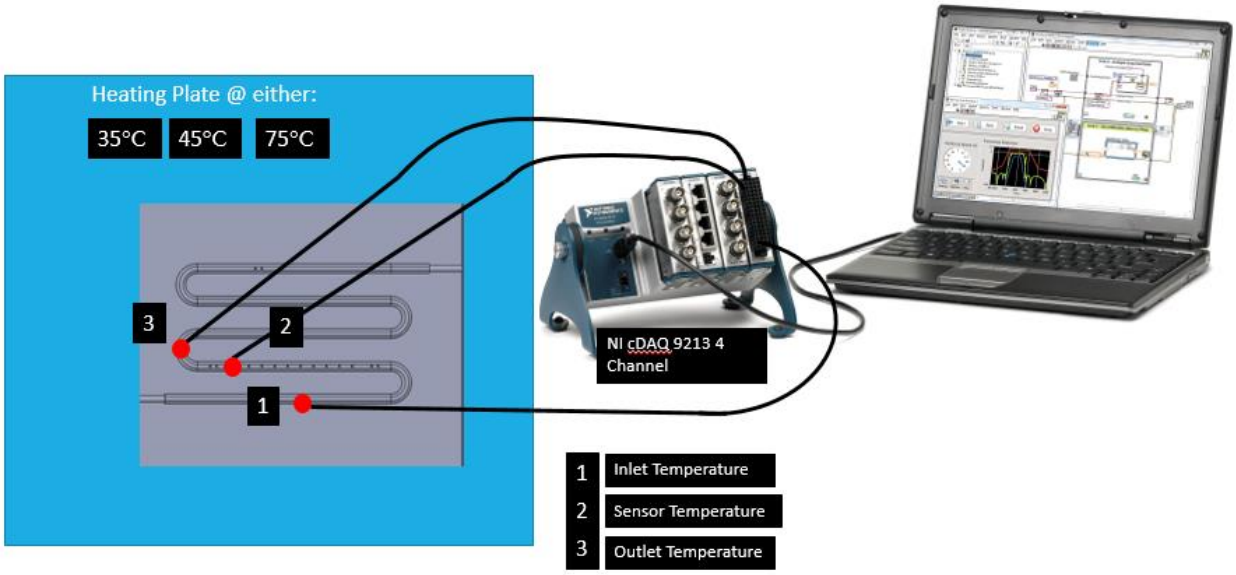

Figure 69: Schematic of Temperature Testing System with Thermocouple, DAQ, and Computer Set Up

6.1.5 Volumetric Flow Rate Testing

Calibration of the syringe pump was necessary in order to ensure no lysis of the sample and also for comparison with other test measures. To do so, our team related several different rotational speed settings to the volumetric flow rate by recording fluid flow through a known section of channel. To test this, first a capillary tube was weighed and then placed onto the analyzer pin. The inlet of the capillary tube was sealed with wax to allow for an air tight seal. Afterwards, 100 µL was allowed to be pulled in by the stepper motor into the tube, and the cartridge was weighed again. Using the density of water (1 g/cm^3) in addition to the change in weight, the volumetric flow can be calculated over the known time. After preliminary tests were performed with the capillary tube, the process was repeated with a sealed cartridge.

6.1.6 Micro-Mechanical Damage Testing

Due to the delicate nature of erythrocytes, turbulence and contamination are two major issues in protecting sample integrity. Thus by passing a rice starch and water mixture through our cartridge, we can characterize the reduction in rice granule size or presence of foreign material in output mixture. This is significant in that mechanical deformation of the granules may indicate potential irreversible damage to RBCs when testing the system with real blood samples. This first was accomplished by

using a benchtop syringe pump (Harvard Apparatus Infuse/Withdraw syringe PHD 22/2000 pump) infusing 10 mL fluid at a flow rate of 20, 30, 40, and 50 µL/sec through the chip. The rice grain size was characterized before and after passing through the chip using a similar method previously outlined in *Section 7.1.1*. The difference between respective minimum and maximum diameters taken from before and after passing the fluid through the cartridge was calculated, and if the difference greater than two standard deviations, the system was determined to be mechanically damaging the sample enough to potentially lead to lysis in real blood samples. *Figure 70* shows the experimental set up.

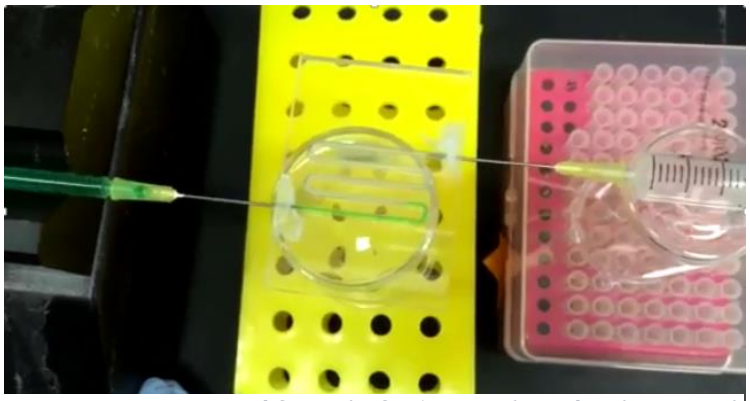

Figure 70: Experimental Set up for Studying Mechanical Deformation of Sample

Additional information may be provided regarding turbulence causing portions by running flow simulations in SOLIDWORKS CFD. An inlet pressure of 330 Pa was used as an initial boundary condition. Areas with pressures greater than 330 Pa and velocities of 6.36 mm/sec

6.1.7 User Interface Peripheral Testing

With the device in use, it was beneficial to the operator to have a relay mechanism that would display the status of analysis process any given point in time. Thus, our team devised an interface to notify the user if the cartridge was correctly inserted, if the cartridge was reading the sample, and if the cartridge was ready to read another sample. The red LED was intended to denote when the system was ready for cartridge insertion, the yellow LED for when the system was reading the sample, and the green LED for when the system had completed the reading and was ready for cartridge removal (please see *Figure 71*). Further expansion of this testing protocol extended to an LCD (Liquid Crystal Display) screen that would relay testing status also.

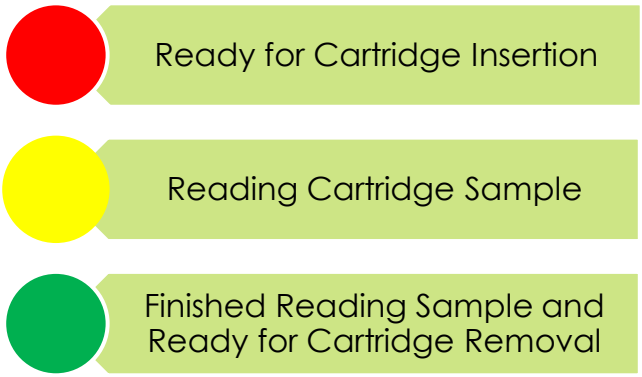

Figure 71: LED array and corresponding system status

6.2 Testing Results

6.2.1 Rice Starch Characterization

Rice starch, as noted previously, was considered a potential blood substitute for preliminary testing procedures due to its cost and availability. We investigated the potential of this idea by determining the ratio of rice starch to distilled water needed to achieve a viscosity similar to that of blood. Additionally the grain size of the rice starch was characterized through the use of a microscope and the ZEN Imaging Software.

6.2.1.1 Viscosity Characterization

After experimental determination, we found that the viscosity of rice starch in water was highly dependent on both rotational speed of the viscometer as well as the ratio of rice starch to water as shown in *Table 9* . In order to reach a desired viscosity of 3.1 cP to mimic that of high hemocrit blood, we varied both rotational speed and rice starch concentration. Testing at a RPM of 200 with a ratio of 0.8377 g of ice starch to 500 μL of water, we found the viscosity is approximately 3.11 cP with a standard deviation of 0.095. Distilled water was found to have an average of 2.933 cP with a margin of error at 0.046188 under the same conditions. Additionally, we found that these mixtures have very good potential for sample mechanical testing of our system due to the clumping behavior of the rice granules under higher seeding concentrations. We eventually selected a ratio of 0.2 g to 500 mL was used in future protocols studying the mechanical deformation of RBCs or rice granules at a RPM that more resembles our intended fluid flow rate while providing a seeding rate best for imaging.

Table 9: Viscosity of Different Rice Starch Mixtures at Different RPM (cP)

Rice Starch/ Water	**0.8277 g/500 mL**			**0.5050 g/500 mL**			**0.4093 g/500 mL**			**0.2001 g/500 mL**			**Distilled Water**		
200 RPM	3.02	3.11	3.21										2.96	2.88	2.96
100 RPM	2.16	2.28	2.22	2.28	2.25	2.22	2.28	2.31	2.31	2.28	2.25	2.28	1.20	1.20	1.20
60 RPM										1.20	1.2	1.20	1.20	1.26	1.32

6.2.1.2 Granule Diameter Characterization

Our final results for rice granule diameter have been summarized in *Figure 73*. We found grain sizes to be 5.993 μm in diameter on average, with a standard deviation of 2.257. Red blood cells range in 6-8 μm [], and thus we found that our mixture was of an adequate size for preliminary testing. Additionally, we observed that the rice starch did clump into bodies that were approximately 8-12 μm depending on our seeding density as shown in

Figure 72, mimicking the similar behavior observed with blood cells. With this in mind, we proceeded to use this rice starch mixture in any of our testing procedures in which the structure or viability of blood cells were of interest to us.

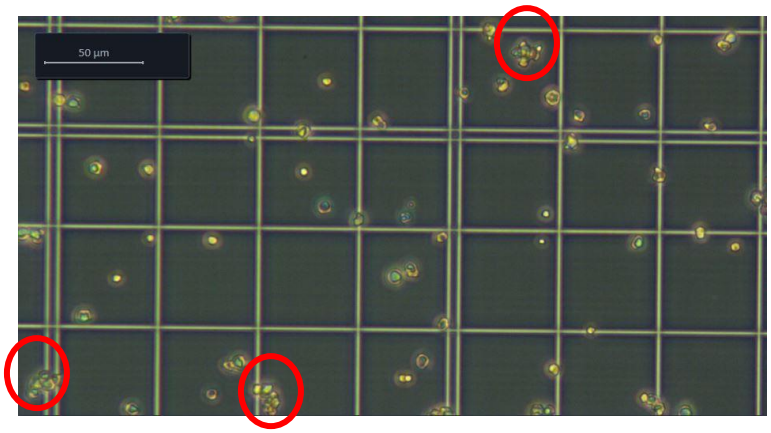

Figure 72: Circled Rice Clumps found in Sample

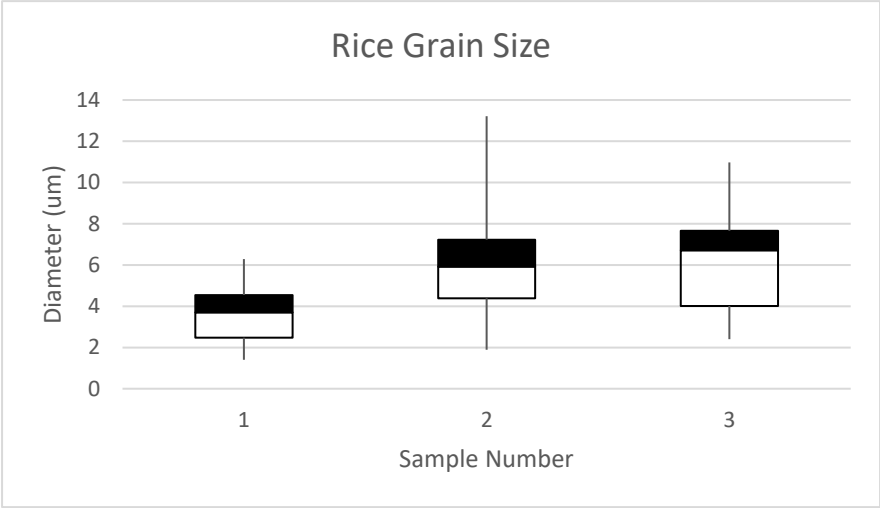

Figure 73: Characterization of Rice Grain Size over Three Trials

6.2.3 Characterization of Cartridge Seal

The team also conducted tests to study if epoxy resin was an adequate binder for the two plates in order to achieve a water tight seal. Following the protocol, we obtained images of the sensors before and after filling the channels as shown in *Figure 74* and *Figure 75*.

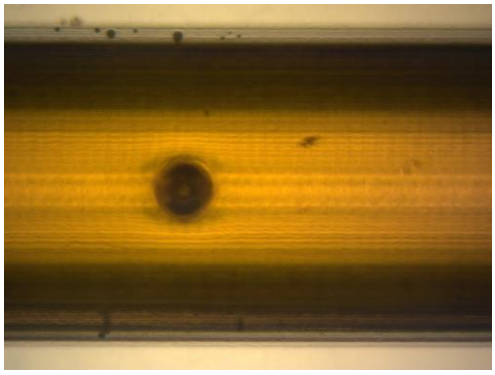

Figure 74: Microfluidic Cartridge with Channel Filled with Fluid

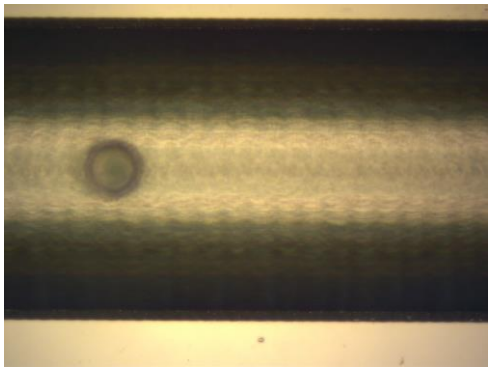

Figure 75: Microfluidic Cartridge with Channel without Fluid

We gathered data for all 9 sensor holes and determined any areas of leakage through the identification of the RGB values on either side of the channel. These findings are shown on *Figure 76*. No differences greater that 18 (a value we had experimentally determined as previously mentioned in the protocols) were found, nor were there any areas of prominent leakage identifiable to the eye. The statistical analysis can be found in *Figure 76*.

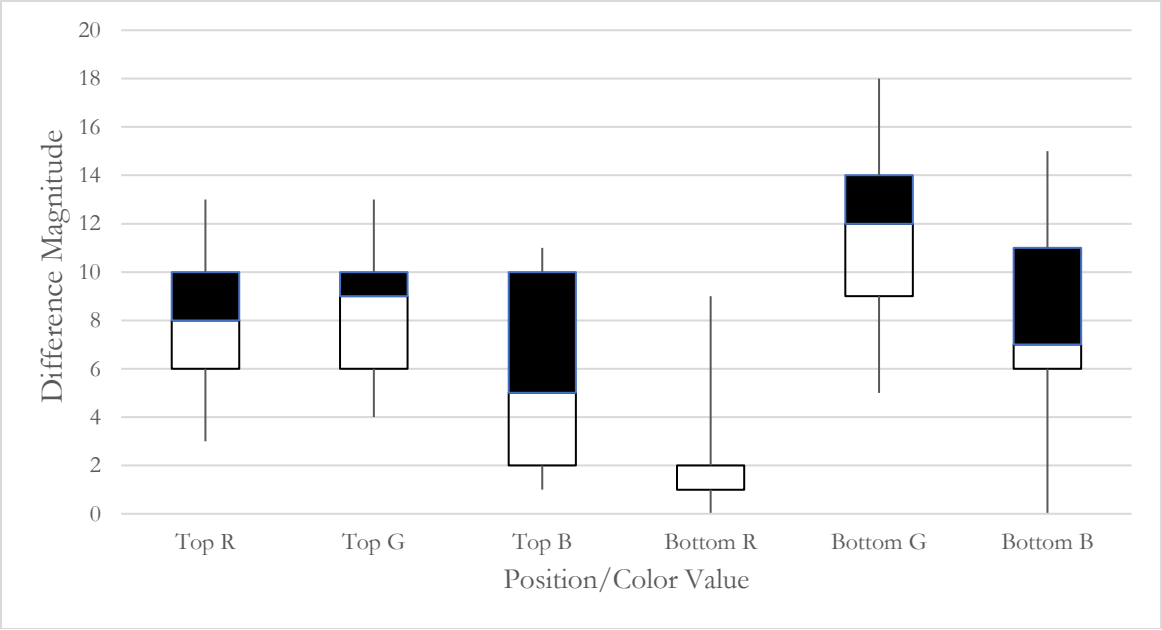

Figure 76: RGB Difference Magnitude Before and After Filling the Cartridge

6.2.4 Vertical and Horizontal Orientation Test Results

With our cartridge successfully sealed, we also wanted to test the fluid behavior once a sample has been introduced into the cartridge. By observing for fluid leakage when the cartridge was turned along the bottom most widest edge as shown in *Figure 77* at 90°, 65°, 45°, and 0°, found the fluid to remain stationary within the chip unless excessive shaking was exacted.

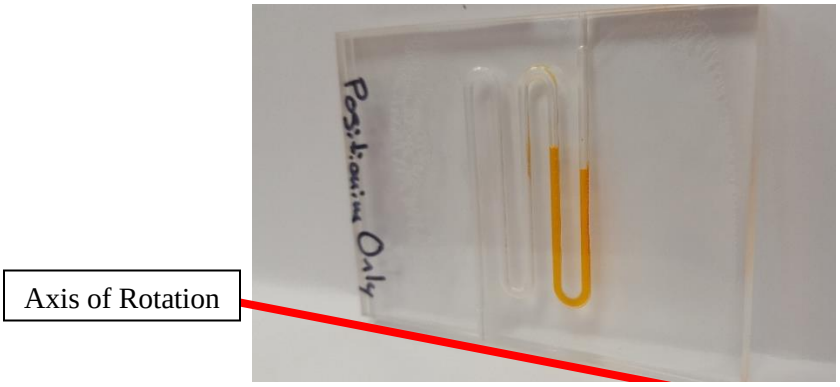

Figure 77: Cartridge with Y axis of rotation

Similarly, we performed the same conditions of testing with the cartridge rotation along the X axis and did find the fluid to have moved through different sections of channel, as shown in *Figure 78*. Due to the extended channel lengths, no fluid leaked out of the cartridge despite the number of turns in either direction except with excessive shaking or force. This allowed us to show that our chip could limit biohazard exposure to the user under prescribed conditions. As a safety measure, we would like to suggest a cap or one way valve to prevent fluid spilling in cases of excessive shaking (please see the Recommendations and Future Considerations section for more details).

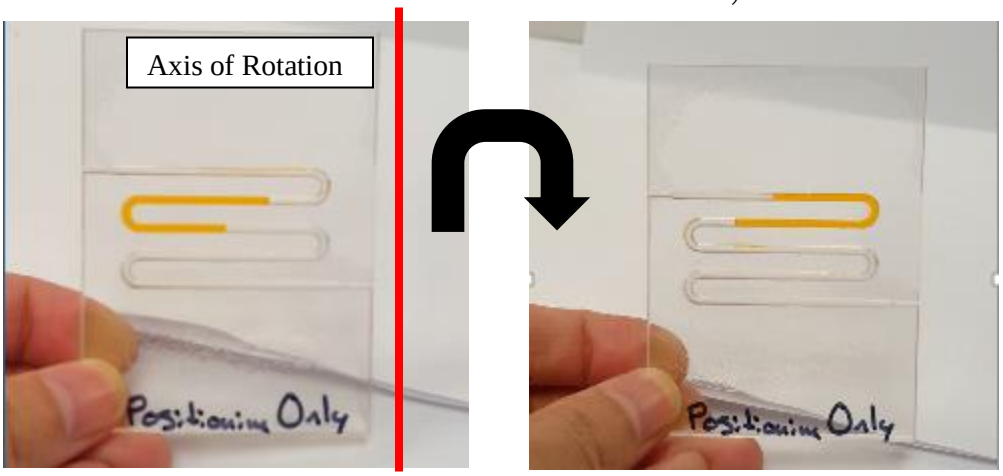

Figure 79: Original position of chip with X axis of rotation

Figure 78: Position of the fluid after rotating the chip 90° to the right along the X axis

6.2.5 Temperature Control

Figure 80 displays the cartridge areas at which at the thermocouples were placed at during test. *Figure 81, Figure 82,* and *Figure 83* summarizes the temperature of the water passed through the chip while on the heating plate over the course of 300 seconds depending on fluid placement. Each line shows the temperature that the heating plate was set to (35°C, 45°C, and 75°C). Time necessary to reach 37°C was noted for each curve if it was under 120 seconds.

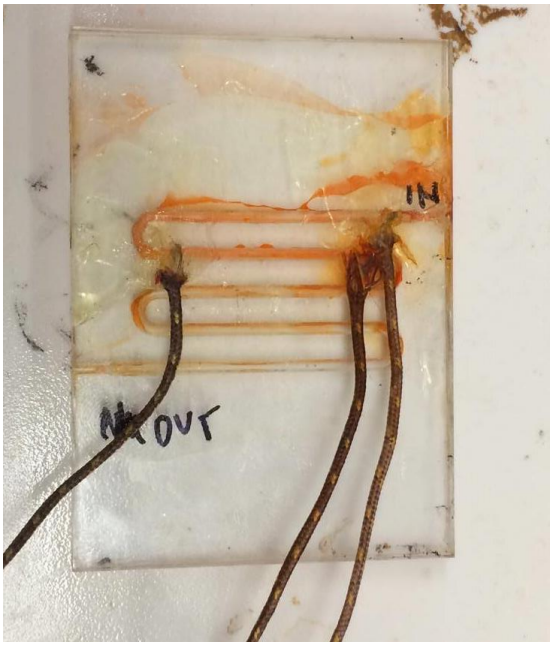

Figure 80: Cartridge with Thermocouples Inserted at Desired Channel Locations

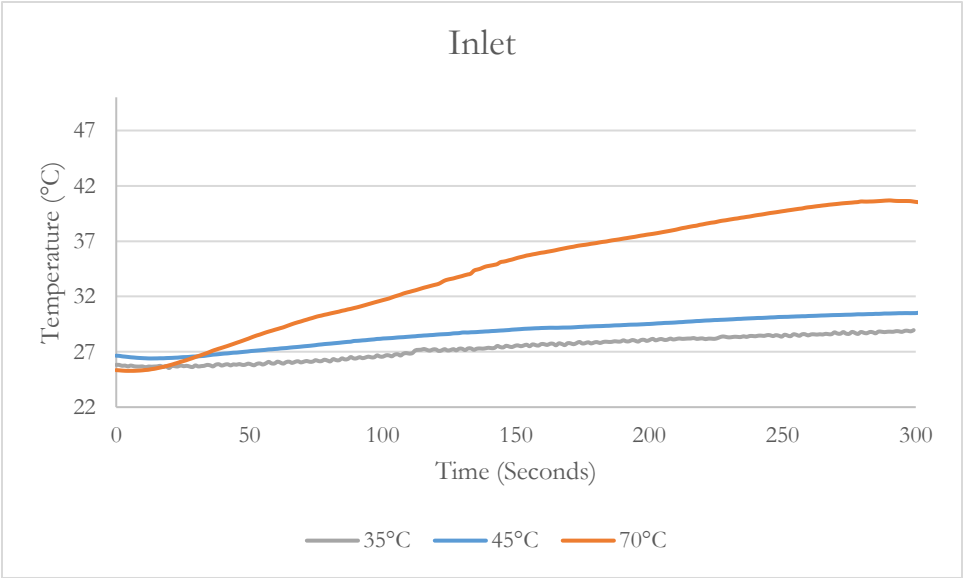

Figure 81: Thermocouple Reading over 300 Seconds at Inlet

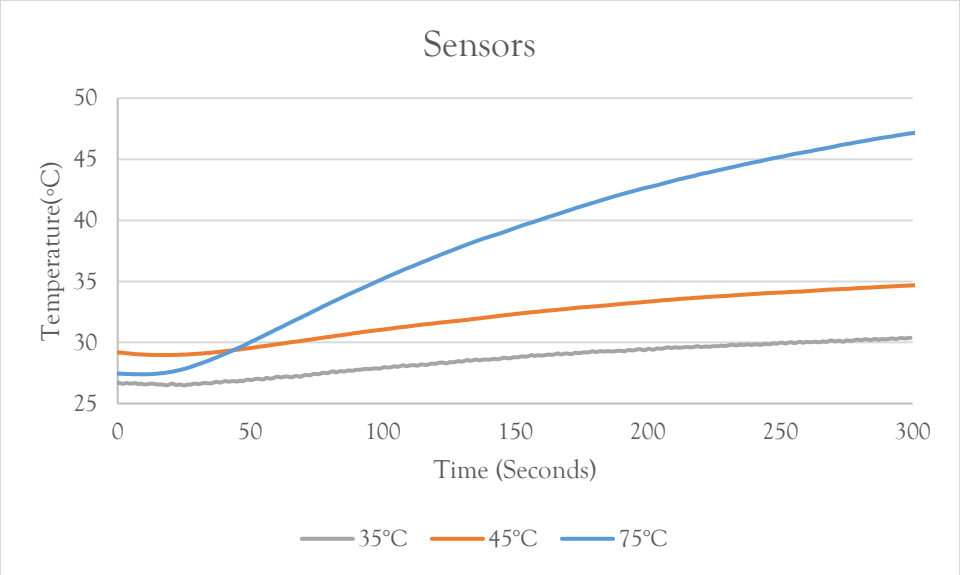

Figure 82: Thermocouple Reading over 300 Seconds at Sensors

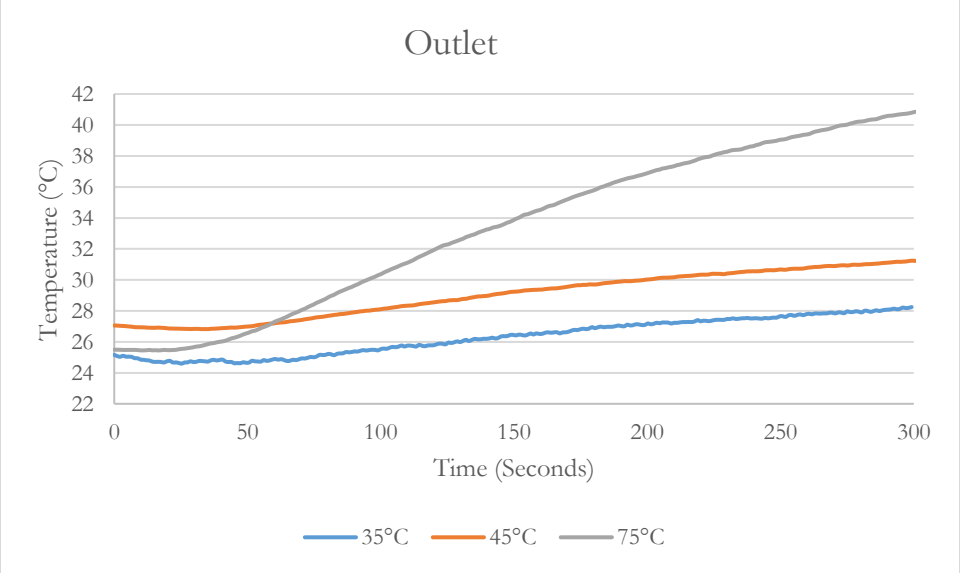

Figure 83: Thermocouple Data over 300 Seconds at Outlet

The chip was determined capable of heating the test fluid contained in a chip previously sitting ambient room temperature to 37°C. However, in order to achieve this temperature within a 100 second limit we had previous determined in order to stay within the 3 minute testing period, a temperature of 75° must be applied. This need for such a high temperature is necessary due to the low thermal conductivity of the PMMA plastic used for the chip. One potential remedy for this issue is to allow for a metal wire pass through the cartridge body and sit closer to the channel to act as a heat conductor. This idea will be explained in detail in the Recommendations and Future Considerations section of this report.

6.2.6 Volumetric Flow Characterization of the Stepper Motor Syringe Pump

We first calculated the expected step rate of the stepper motor that would correspond the anticipated volumetric flow rate based on the volume of the 1 mL syringe used in the pump system. Table 10 summarizes the rotation step rate and the motor's corresponding rotational speed, calculated volumetric flow rate, and time necessary to move 100 μL of fluid into the channel length of the same volume. It should be noted that the maximum volumetric flow rate our motor was able to accomplish was 15 μL/sec, much lower than that used by IL in their GEM systems.

Table 10: Step Rate Correlated to Flow Rate and Time

Step Rate	**Rotational Speed (revolutions /second)**	**Volumetric Flow Rate (μL/sec)**	**Time to move 100 μL (sec)**
Full Step	1.12	16	6.25
Half Step	0.56	8	12.25
Quarter Step	0.28	4	25
Eighth Step	0.14	2	50
Sixteenth Step	0.068	1	100

With this in mind, we performed testing on both a 1 mL capillary tube as well as a cartridge with the inlet and outlet seals with clear caulk. For the capillary action, we varied the step rate in order to see evidence of volumetric flow rate variation in response step rate variation, and the results are shown in Table 11.

Table 11: Summary of Volumetric Flow Rates through Capillary Tube at Different Step Rates

Run	**Step Rate (rev/sec)**	**Empty Tube Weight (g)**	**Tube with Liquid (g)**	**cm3**	**Fluid Passed (μL)**
1	2000	0.375	0.44	0.065	65
2	2800	0.369	0.439	0.07	70
3	2800	0.369	0.429	0.06	60
4	2800	0.368	0.428	0.06	60
5	3000	0.375	0.47	0.095	95
6	3000	0.375	0.465	0.09	90
7	3100	0.374	0.474	0.1	100
8	3100	0.376	0.476	0.1	100
9	3100	0.393	0.493	0.1	100
10	3100	0.398	0.495	0.097	97

With this data, it was noted that the step rate of 3100 does allow for 100 μL of fluid to pass into the capillary tube with a high amount of accuracy. We repeated this experiment with the final cartridge design after sealing the inlet and outlet of the cartridge with caulk. For each of this runs, a stead step rate of 3100 rev/sec was used to arrive at the results shown in Table 12. The average flow rate was

found to be 13.38 µL/sec with a standard deviation of 1.11. Additionally, the amount of fluid pulled through was averaged to be approximately 83.83 µL with a standard deviation of 6.11. We found that due to issues with the seal at both the inlet and outlet, this allowed for greater flow rate and variation volume that expected. Nevertheless, this syringe pump was able to pull enough fluid (>65 µL) to be placed over the sensors.

Table 12: Volumetric Flow Rates for Fluid Flow through Cartridge

Run	Initial (g)	After (g)	Difference (g)	volumetric flow rate (µL/sec)
1	25.701	25.79	0.089	14.68646865
2	25.706	25.784	0.078	12.97836938
3	25.695	25.784	0.089	14.4012945
4	25.693	25.783	0.09	13.88888889
5	25.697	25.774	0.077	11.9379845
6	25.689	25.769	0.08	12.42236025

6.2.7 Characterization of Micro-Mechanical Deformation of Rice Granules

Sample integrity testing was performed both with a calibrated benchtop syringe pump as well as with our stepper motor syringe pump after volumetric flow validation tests were performed in order to ensure proper calibration.

6.2.7.1 With Benchtop Syringe Pump

As previously mentioned, we performed our testing the infusion setting of the syringe pump for our initial tests. After characterizing the starch sizes before and after passing it through the cartridge at 20, 30, 40, and 50 µL/second, we arrived at the diameter ranges shown in *Figure 84*

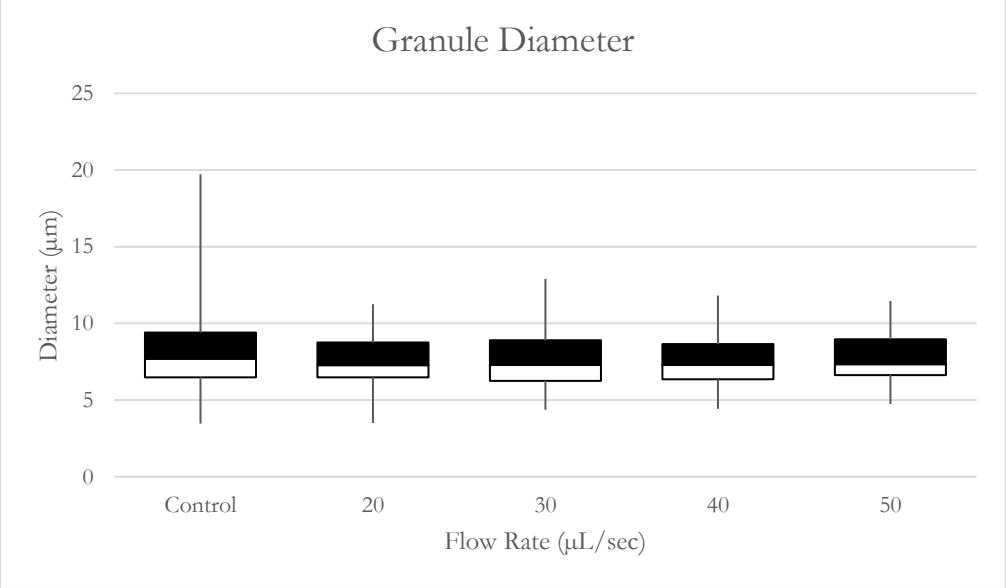

Figure 84: Rice grain diameters before and after passing through the cartridge

We performed a confidence interval for the comparison of the differences in the mean diameter of the control sample compared to the mean diameter of the testing sample of each flow rate. We found that 20 µL/sec and

30 μL/sec show no statistical significance (P<0.1) in diameter change before and after running the sample through the fluid, and thus there was no drastic deformation in the sample. However, we found that running the fluid at both 40 μL/sec and 50 μL/sec did show a statically significance in diameter size change, and therefore determined that 30 μL/sec is the maximum fluid flow rate to run our sample at while still preserving its integrity.

6.2.8 Verification of User Interface Peripheral Functionality

We programmed our Arduino UNO board to allow the LEDs to convey to the user the status of the testing process. We successfully implemented this user display as shown in *Figure 85*. The red LED denoted pumping, the yellow denoted that the sensor was currently taking a measurement, and the green denoted that the device had completed retrieving the results.

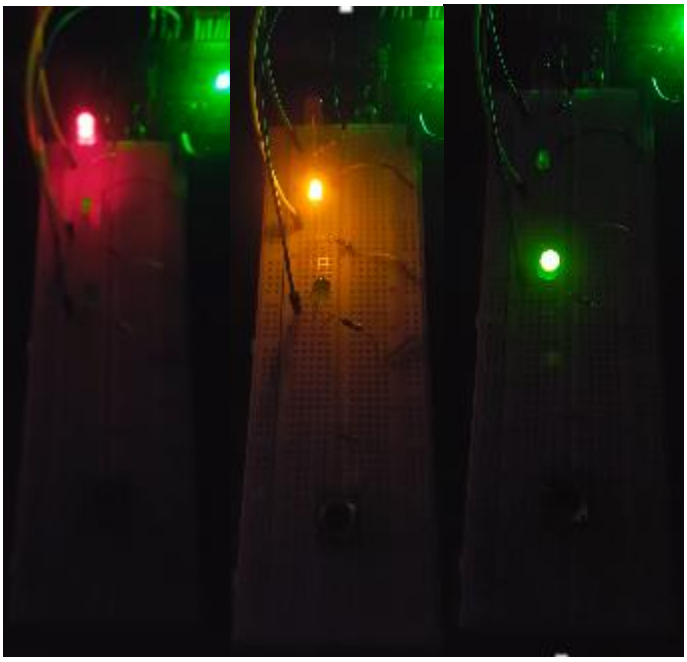

Figure 85: LEDs denoting what step in the process the device was currently in

7. Discussion and Design Validation

7.1 Design Validation Evaluation

The two major areas of focus in this project was first to create a microfluidic cartridge with the potential of maintaining conditions for optimal IL sensor readings, and second to create an affordable fluid positioning system that was able to accurately create a smooth and continuous withdrawal rate. As we saw in the results, our cartridge is capable maintaining fluid at the desired temperature of 37°C, pass fluid with low damage to the delicate micro particles, and prevent major biohazard exposure to the user under ideal conditions.

The following charts highlight in green the client needs that our device have been validated capable of performing. The yellow highlighted blocks indicate client needs that have not yet be verified through testing due to lack of time and resources.

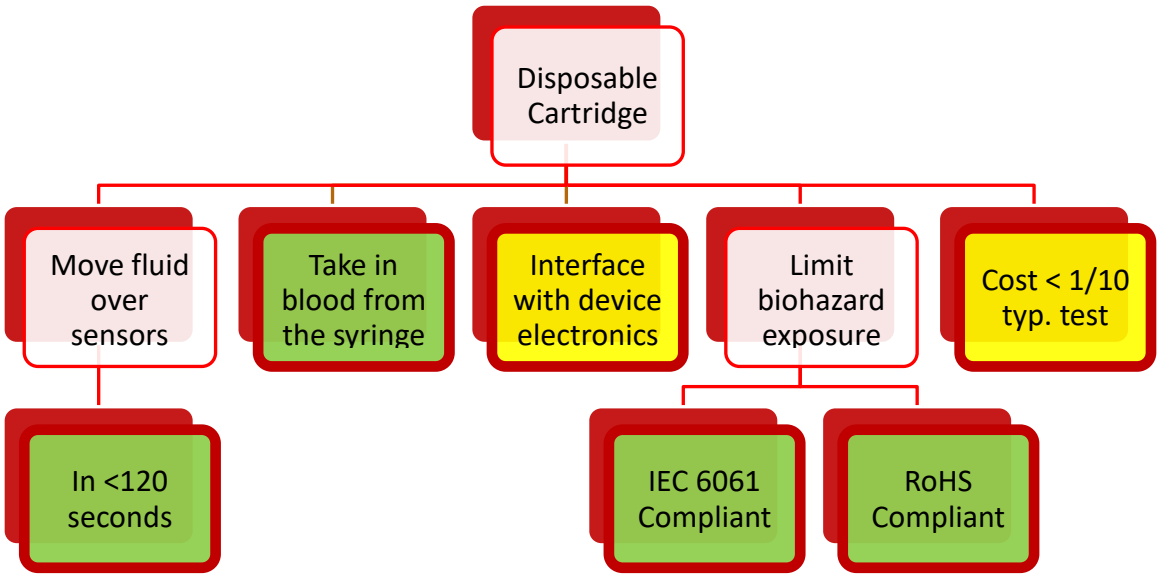

Figure 86: Design Validation Tree for the Disposable Cartridge Objective

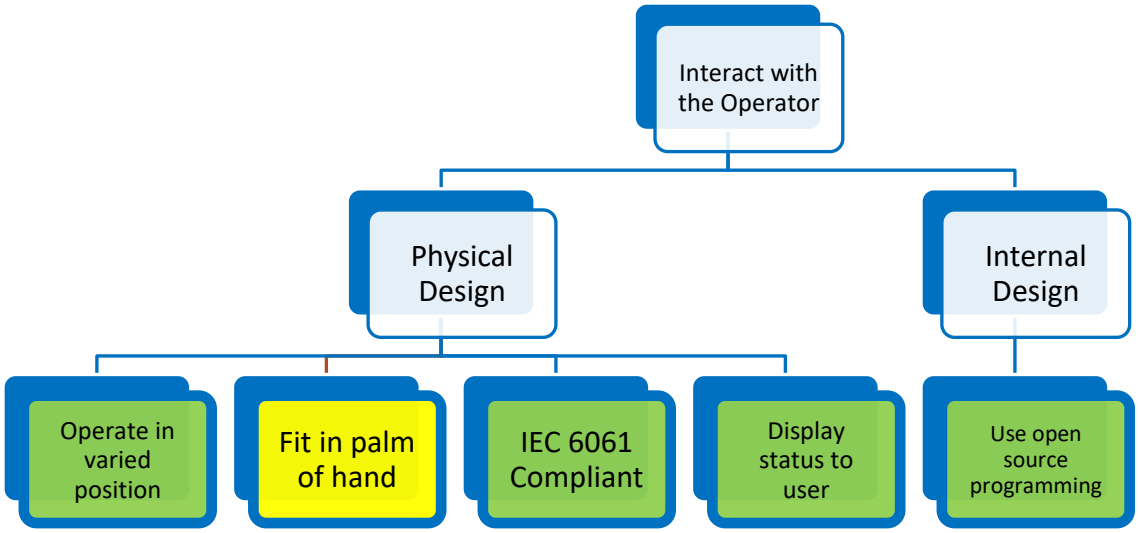

Figure 87: Design Validation Tree for the Operator Interaction Objective

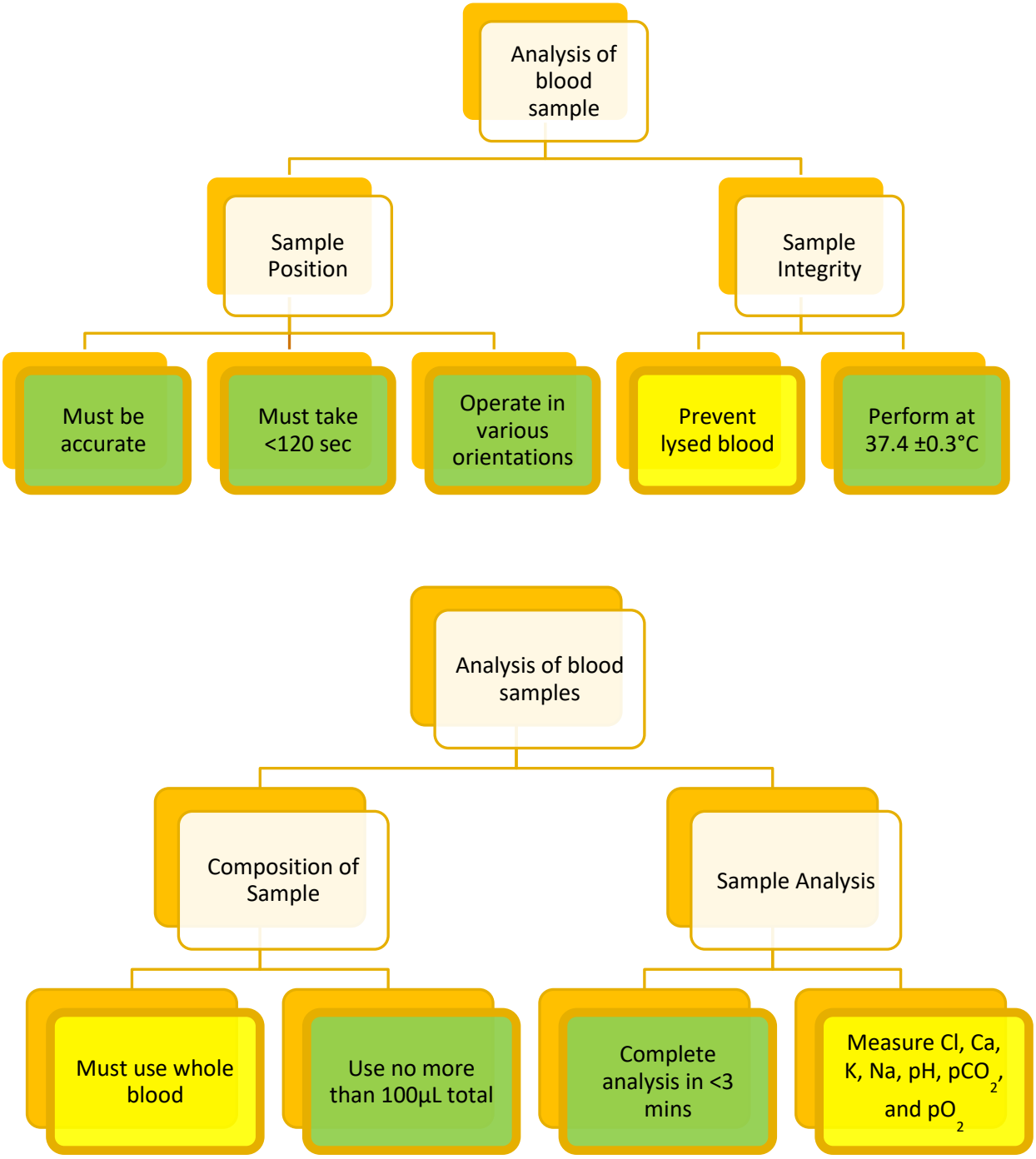

Figure 88: Design Validation Tree for the Blood Analysis Objective

As we saw in the results, our cartridge is capable maintaining fluid at the desired temperature of 37°C, pass fluid with low damage to the delicate micro particles, and prevent major biohazard exposure to the user under ideal conditions. From a manufacturing standpoint, our current methods would not be suitable for mass production. However, several slight modifications to the fabrication method can

easily bring our design into production, as discussed in the Recommendations and Future Considerations portion of this report.

Our current design can be assumed to be able to fulfill the following client needs although this have not been verified through testing:

- The device shall complete analysis on whole blood.
- The device shall operate in any handheld orientation.
- The disposable component shall interface with the device electronics to support sensor function.

Due to the limited time and resource constraints, we were not able to perform the protocols that would have allowed us to show that our cartridge indeed did satisfy these requirements. However theoretically, our system has been designed to be capable of accomplishing these needs. We have tested for potential sheer strains that may damage the delicate red blood cells through using a rice starch mixture, suggesting that our device is capable of performing tests with whole blood while minimally damaging the red blood cells mechanically. Additionally, a heparin slug may be used in order to prevent clotting and help maintain the ideal fluid viscosity over time. The air tight seal at the inlet and outlet after chip insertion and using the syringe pump as the only mode of fluid flow will allow our device to maintain and manipulate pressure and thus allow it to operate in a variety of upright positions. Finally, we have accounted for both electrolytic and positioning sensors to easily be placed within the channel base. It is our hope that IL will continue with this project and study the potential of our system to satisfy these requirements.

However it is noted that our overall design leaves much to be considered. Although our chip is capable of maintaining these set parameters (i.e. temperature, containment, etc.), it has not yet be verified with true blood samples. Additionally, instances where misuse of the device have not been accounted for, and thus additional safety mechanisms may be necessary. Suggestions for potential refinement have been enclosed in this report under the Recommendations and Future Considerations section.

7.2 Economics

With the need for fast turnaround times for blood gas analysis results especially in healthcare settings, such as the ER, critical decisions must be made fast based on the diagnostic information. The wait time for blood labs running massive batches of samples adds to the necessary wait time, which in turn may compromise medical advice and decisions made under very tight time constraints. The POC can be done at the bedside of the patient with results in a matter of minutes, which in turn saves time, money, and lives. With the cost of healthcare in America rising to expensive price tags [74], finding low-cost yet still reliable, accurate, and effective means of providing diagnostic information can affect the economics of the health care system. Additionally, the competitive cost per cartridge test may appeal to insurance agencies as well as individuals paying for their medical costs up front when compared to $100 per test insured, or $1,500-$3,000 per test uninsured.[75]

7.3 Environmental Impact

Our project involves creating a *disposable*, microfluidic cartridge capable of performing blood gas analysis. Acrylic plastic, specifically PMMA, has strong bongs and will not decay for decades therefore is not biodegradable [76]. According to PMMA Acrylic Sustainable Solutions, a manufacturer that operates under the European Chemical Industry Council, PMMA can be recycled via pyrolysis, which

PMMA is heated to high temperatures in the absence of oxygen or depolymerization to break PMMA down to MMA monomer [77]. However, this process of heating up the PMMA required substantial heat and energy and may release toxic particles into the surrounding environment [76]. Therefore, even though our cartridge can be recycled, it may not be saving energy. However, manufactures make PMMA through energy efficient processes because it is a lighter material compared to other plastics and metals, and it has been shown that MMA monomers can be recycled to high purities of PMMA for reuse. Therefore an avoid effort to recycle our PMMA cartridges using a biohazard recycling program often seen in laboratory and healthcare settings may reduce the environmental impact of our analysis system.

7.4 Political Ramifications

The concept of a POC blood analysis device allows for affordable, fast, and easily accessible diagnostic information. This could have an impact on the health care systems in countries where patients do not have the financial means or geographical access to conventional blood work laboratories. This device can give healthcare professionals easily accessible means to make critical care decisions, even in compromised healthcare settings such as field hospitals with limited power or in hard to reach locations. These doctors would be able to get the blood analysis information they need in order to make accurate diagnostics for the patients.

7.5 Ethical Concerns

By providing quicker diagnostic results rather than waiting for lab results, healthcare professionals can make critical care decisions without a long delay that may compromise the health of the patient under time sensitive conditions. Therefore health concerns can be addressed quicker and can help more patients, allowing for better care not only in a hospital setting but also for potential home care applications. Ethical concerns regarding device use center on protecting personal medical information, such as test results produced by our device, and also on providing accurate diagnostic information from which doctors may objectively make informed decisions.

7.6 Health and Safety Issues

This product measures the gas values from a blood sample, and therefore poses the potential risk of biohazard exposure to the user. With this in mind, we performed various testing protocols to assure that the microfluidic cartridge as well as the handheld analyzer limits the amount of biohazard and blood exposure. However, the blood samples should be handled with care and the device used as intended in order to ensure the safety of user from a manufacturing standpoint.

7.8 FMECA Scoring

At the conclusion of our testing and design validation, we performed Failure Modes, Effects, and Criticality Analysis or FMECA on our overall system in order to characterize potential failure points of the device as well as how it would affect the safety of both the device and consumer. Each device component was evaluated for different modes of failure such as mechanical, chemical, or electrical, and evaluated for potential causes of each failure. Then, the potential likely hood of occurrence, severity, and detection of each type of failure was assigned a scoring from 1 to 10, with values corresponding to impact values outlined in Table 13 to Table 14. Our final FEMCA scoring

Table 13: Occurrence Scoring Values

1	Extremely unlikely
2 3 4	Unlikely/low probability
5 6	Likely
7 8 9	Highly likely/ high probability
10	Extremely likely/almost certain

Table 14: Detection Scoring Values

1	Extremely likely
2 3 4	Highly likely
5 6 7	Likely
8 9	Unlikely
10	Extremely unlikely

Table 15: Severity Scoring Values

1	No effect on customer or process
2 3	Slight customer annoyance/operator inconvenience
4 5	Moderate customer annoyance/minor in-house cost
6 7	High degree of customer annoyance/major in house cost
8 9	Failure to provide the service/loss of production capability
10	Catastrophic – safety/health implications

No.	Component or Function	Type of Failure	Failure Mode or Defect (Short/Long term Defects)	Failure Cause	Local Effect	Potential System Effect	OCC	SEV	DET	RPN
1	Syringe	Mechanical	Loss of lubrication fluid/High friction	Break in rubber plunger	Fluid movement inaccurate or nonexistent	Does not deliver blood accurately over sensors	3	10	3	90
		Mechanical	Does not interface with cartridge outlet/breaks device	Broken syringe tip/occlusion of tip/improper insertion of cartridge/failure of cartridge	Fluid movement inaccurate or nonexistent	Does not deliver blood accurately over sensors	3	10	3	90
2	Stepper-Motor	Electrical	Permanent damage to device.	Overheating/ short circuit	Device and surroundings functionality compromised	Cause the electrical components to malfunction/be useless	2	10	3	60
		Mechanical	Loss of torque	Wearing out	Fluid movement inaccurate or nonexistent	Does not push/pull plunger for ejection/suction (negative pressure) needed for the system.	3	10	5	150
3	Pulling Plates	Mechanical	Does not pull syringe	Wearing out	Fluid movement inaccurate or nonexistent	Does not deliver blood accurately over sensors	5	9	5	225
4	Micro-controller	Electrical	Cease functionality of electrical peripherals	Incorrect wiring or broken pin	Nonfunctional peripheral	System failure/does not work if peripheral depends or is depended on other	7	8	1	42

						components of the system				
5	Battery	Chemical	Cease functionality.	Lifespan	Device would no longer turn on	System failure/no power	3	10	3	90
		Chemical	Cease functionality.	Damage to battery	Leak battery fluid and harms user/contaminates system	System failure/chemical hazard/ components no longer functional	2	10	3	60
		Electrical	Cease functionality.	Short circuit/inappropriate wiring	Electrical components burn out microcontroller	System failure/electrical components no longer functional	2	10	3	60
6	Sensors	Electrical	Inaccurate readings	Pins not properly printed and connect to sensor	Inaccurate or no data	Incorrect results displayed	2	10	5	100
		Material	Inaccurate readings	Improper reagent hydration prior to use	Inaccurate or no data	Incorrect results displayed	2	10	5	100
7	Epoxy Adhesive	Mechanical	Cartridge falls apart	Inconsistent sealing	No blood containment	No blood in system/biohazard	2	10	3	60
8	Microfluidic Cartridge	Material	Leakage from Inlet or Outlet	Crack/improper seal	No blood containment	No blood in system/biohazard	2	7	3	42

8. Recommendations and Future Considerations

Although our chip has fulfilled the majority of the client needs, there are still many considerations and improvements that will not only allow the device to be fully functional and ready for manufacturing, but also a strong competitor in the POC blood gas analyzer market. In this section, we outline the future course of action that would allow IL to further refine our design, in particular, cartridge sealing as well as manufacturing techniques.

8.1 Cartridge Sealing

In order for fluid movement to take place, the microfluidic cartridge must have an airtight seal between the two plates and also at the input and output, which can be broken at time of cartridge insertion. Accomplishing this has been a manufacturing challenge for the team due to inconsistent success between sealing different iterations of the cartridge, and thus further research needed to be done into other potential methods of adhesion. Below details three possibilities the team researched into that may create the desired seal without the many issues that arose with our current methodology.

8.1.1 Ultrasonic Welding

Ultrasonic welding, also known as localized welding, is the practice of bonding two separate pieces of the same material to each other through heating the surfaces of the mate parts using ultrasonic energy. In order for this method to be feasible, many conditions must be met: the parts must be made of the same thermoplastic, the melting point must be within tolerances, the material must be homogeneous, and there must be energy concentration points in the design to create and contain surface melt flow, as shown in *Figure 89*.

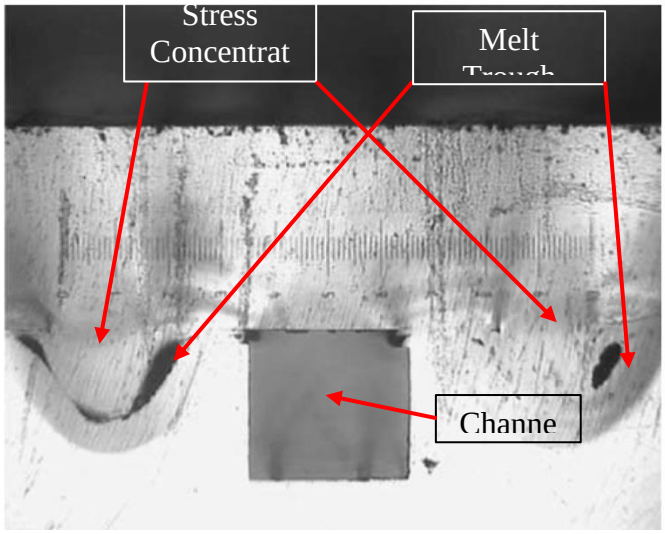

Figure 89: Ultrasonic Welding, showing stress concentrator and trough design for sealing Channel [78]

While this process is quick and can be fine-tuned to seal efficiently, the need for a specialized chip design as well as the inability to bond to different materials does not allow this sealing method to be effective for prototyping or initial stages of production. In the case of the microfluidic cartridge, this was not a feasible small scale option and was not pursued.

8.1.2 Adhesion Bonding

The most versatile of the indirect bonding methods, adhesion bonding, allows the manufacturer to tailor the strength and type of adhesion between to components. With the use of PMMA as the main base material of the microfluidic cartridge, epoxy resin adhesion between the two plates was a feasible solution for the team to pursue. This was due to the local availability of the epoxy and the ease of application.

While this epoxy sealing was adequate for the prototyping stages of the design process, there is a serious need to investigate alternative methods of adhesion bonding. One such method the team found was UV adhesion, in which an adhesive is placed on the contact surface between a part and its positioned mate part. A mask is then placed over the entire set up in order to keep UV light from penetrating the material where a bond is not wanted, allowing for specific placement of bonds. Once the UV light has been shone and the components have been mated, water or a solvent is passed through the channels, removing any excess adhesive [78]. This form of bonding would lend itself well to the microfluidic cartridge designed in this paper and should be further explored.

8.1.3 Pressure Fit

In this final potential adhesive method, the two desired mating components are fabricated with matching protrusion and depressions that will interlock together. The tolerances when making these two parts must be extremely tight in order to allow for an air tight seal to be created. There is no literature on this type of bonding for microfluidic devices, however there is plenty regarding larger scale components. Moving forward, IL may analyze the time and cost investment that would be required to adapt these pre-existing processes.

8.2 Manufacturing Considerations

One of the major determinates of this project's marketability depends on the ability of IL to fabricate this design for mass consumption. The team looked into many of the current fabrication methods on the market and made a product specific overview of each method to facilitate future manufacturing decisions. Each manufacturing process was evaluated on: overall startup cost, cost per cartridge, rate of production, and adherence to tolerances.

8.2.1 Micro Molding

Micro molding is a process in which micron precision of microstructure metallic molds are transferred to polymeric products [79]. This end result can be achieved through many different manufacturing processes including: micro injection molding, hot embossing, and thermoforming. In the next sections each process is detailed and the pros and cons are presented for the manufacturing of IL microfluidic cartridge.

8.2.1.1 Micro Injection Molding

Micro injection molding utilizes the same processes as regular injection molding, just at a miniaturized scale. After the molds are made injection molding takes place in the following three steps: plasticization (inserting the material into the hopper and allowing it to become molten), injection-filling-packing (injecting the material, filling the mold, pressing the molds together forcing a packed fit), and cooling/ejection [80]. This process is depicted below in *Figure 90*.

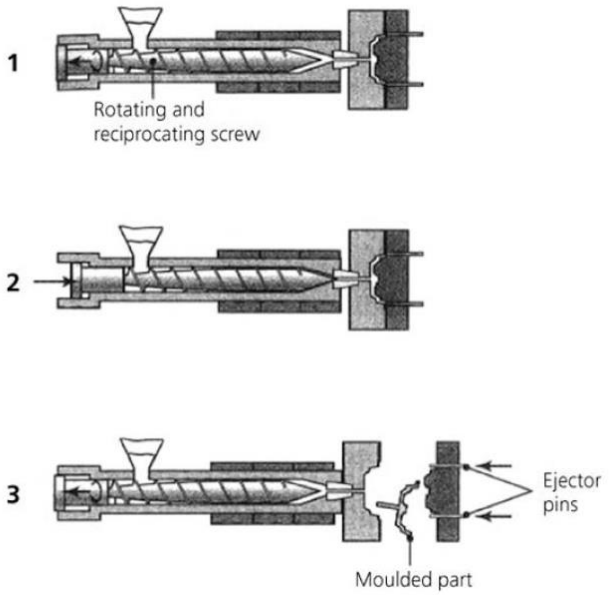

Figure 90: Injection molding Process: 1) Plasticization, 2) Injection/Filling/Packing, 3) Cooling/Ejecting [80]

High production output, low labor costs, high tolerance repeatability, and ease of intricate shape fabrication are just a few of the reasons that injection molding is one of the most used manufacturing methods for polymers. Injection molding allows the manufacturer to create a high volume of nearly identical parts with very little effort after set up is achieved. This form of fabrication allows for very fine tolerances to be achieved which is ideal for microfluidic production [79].

Although this fabrication process does boast of many advantages, the overhead costs for this method of production are considerable, and the creation of the mold for the part and the determination of material type and composition are all considerations that take time and money [81]. The mold creation is an intricate process that both uses the final desired part footprint and the necessary channels for the material as well as the machinery. This process also limits the material to be used due to a very specific set of material properties needed to be successful in injection molding (Liou). The material must have a glass transition temperature, melt temperature, and thermal expansion coefficient within a desired range (Becker). Injection molding also places high shear stresses on the material and creates high inner stresses within the final part due to its rapid flow rate and long path of travel [82].

Keeping in mind the overall shape and construction of the microfluidic cartridge, the creation of internal stresses, especially surrounding the holes for the sensors and positioning pin, would be unacceptable. For this reason, injection molding, while the cheapest of the many options, is not suitable for the creation of this component. This cartridge could be redesigned to allow for this type of manufacturing if overall cost was a huge problem.

9.2.2.2 Hot Embossing

Hot Embossing is a process in which two dies are placed on top of each other with a material in between, heated and then compressed, forcing the material to conform to their shape. The process is shown below in *Figure 91.* Used when the glass transition temperature of a material is not compatible with injection molding, hot embossing is a moderately expensive process this is simpler to replicate and has fewer variable parameters as compared to other methods [83].

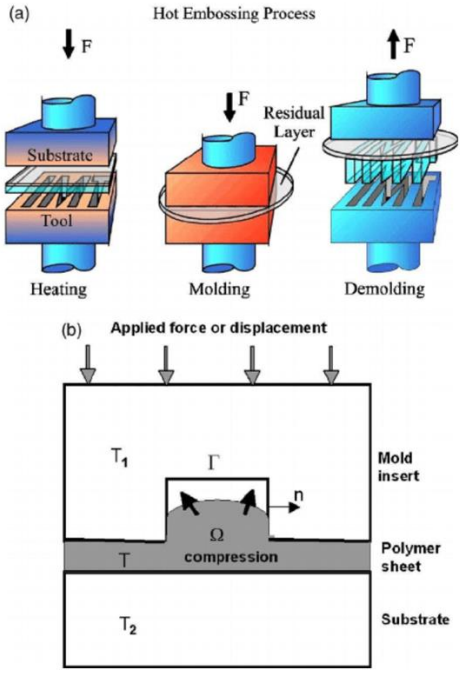

Figure 91: Hot Embossing Process [82]

The advantages of hot embossing include low material flow, limited internal stresses, and low flow rate. This allows for more detailed structures to be fabricated with increased accuracy. The precision in which these process can achieve when run under controlled conditions is greater than that of X-ray Lithography [84]. The material used in this process is also a semi finished product meaning that there is homogenous distribution of the melt over the surface of the tool, filling the cavities in the most efficient manner and shortening the flow path. As seen above in *Figure 91*, longer flow paths mean high shear rates and a creation of inner stresses on the final part, in this way hot embossing manages to avoid these problems. This also makes it ideal for "large area thin components and structures with extreme aspect ratios" [82].

Hot embossing, while boasting of many strengths, does have some drawbacks when it comes to material choice. With proper force and care hot embossing can lay micro patterns on a silicon wafer [84]. However, the manufacturer is limited to materials with small thermal shrinkage coefficients in order to maintain structure tolerances once the part is cooled. Other limitations are the glass transition temperature, rigidity, and overall deformation of the material. Considering the decrease of internal stresses and the increase of tolerances for thin components, hot embossing seems to be an ideally suited production process for the fabrication of this microfluidic cartridge.

8.2.2 Micro Thermoforming

Known today for the creation of hollow plastic structures such as bottles and cups, thermoforming is an efficient means of fabricating fluid containment devices. The process of micro thermoforming has become revolutionary within the microfluidic sphere due to the fact that this process can takes a polymer in its entropy-elastic state and can create films of polymers as small as 2μm thin [82]. Micro

thermoforming even has the ability to embed sensors within the formed component, this is in *Figure 92* [85].

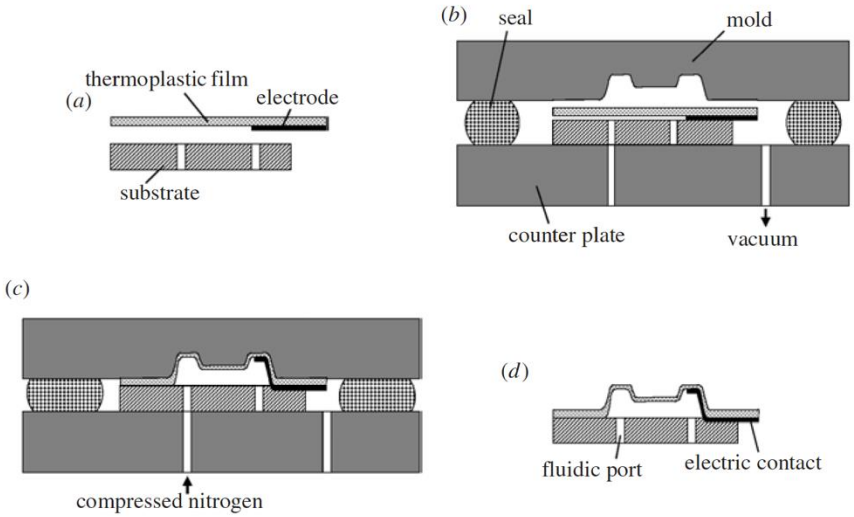

Figure 92: Micro Thermoforming a device with embedded sensors [85]

This process can even be replicated on a less rigid material enabling the creation of a roll of fluidic components, shown in *Figure 101.*

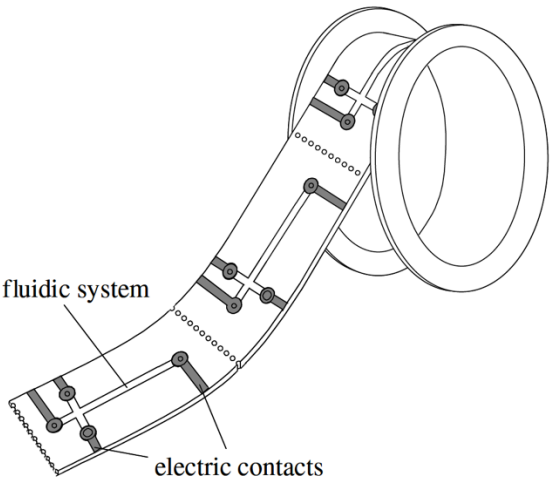

Figure 93: :Micro Thermoformed Microfluidic device with embedded sensors on a roll [85]

There are many different manners in which to preform micro thermoforming, below is an image that details four of the most common methods used.

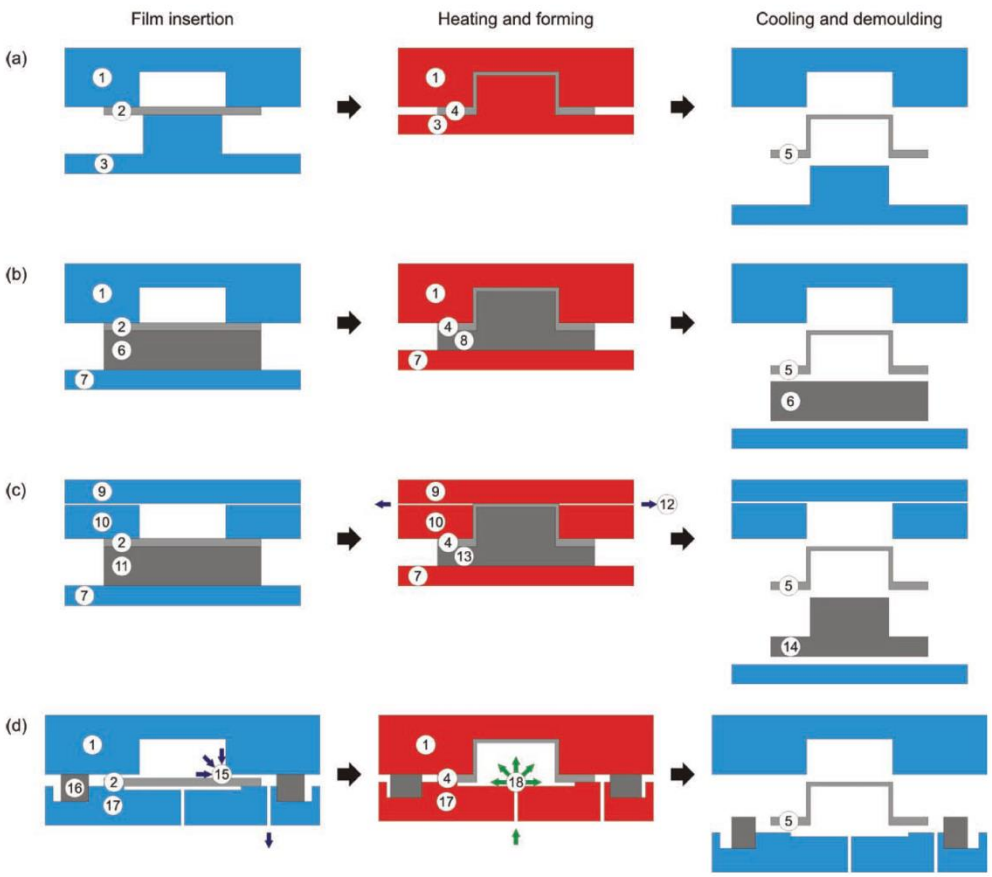

Figure 94: Variants of the microscale thermoforming process (using the example of negative forming versions): (a) Forming with a matching counter tool – micro matched-die molding, (b) with an elastomeric counter tool, (c) with a softened polymer – micro back molding, and (d) with compressed gas – micro pressure forming (simplified schemes); blue and red corresponds to cold and heated, respectively. [85]

Although this process resembles hot embossing, it is drastically different when it comes to scale of final parts. Micro thermoforming can produce extremely thin walled components without the need of a thicker base layer for protrusion. This process also uses the material when it is still in a semi solid state, where as hot embossing manipulates the material to a viscous or even liquid state [86].

Overall, micro thermoforming is still a relatively new method of manufacturing that allows for many innovative techniques. It is more expensive than hot embossing and micro injection molding, but the rewards of not needed further processing steps can offset these costs. For the case of the microfluidic cartridge in this paper there are both pros and cons to using this method of manufacturing, the first being the need for further research into the exact steps necessary to utilize this process and the costs associated with that research. This process seems like an ideal method, but with little information further speculation cannot be made as to how it can be implemented large-scale.

8.3 Electronic Interface

One of the project requirements was that our disposable cartridge should be able to interface with IL's current sensor technology. Therefore the next step in the project is to incorporate the electrolyte and blood gas sensors into the microfluidic cartridge and set up the pins in order to collect the data.

The sensor pins would then connect to the microcontroller to send the measured readings and perform the data analysis. This would require the pre-placement of reagents over the sensors before testing as well as ensuring correct sensor readings by establishing a self-regulating standard reagent the system could compare to.

The system also needs to integrate the position sensors places along the channels. The feedback from these pins would be able to better control the stepper motor and act as a preventive measure against over drawing of blood or reagents into the syringe pump, therefore minimizing biohazard exposure to the device and user. Additionally, the positioning sensors would act as a bubble detector in case that air was trapped while introducing the blood sample into the system, allowing the motor to either reposition the blood to avoid the air bubble or if this is not possible, disregard the sensor reading over which the bubble was over. All of these sensors, both position and analytic, may be controlled from a custom self-contained microcontroller that would read in these values and also control the motor.

Finally, the user interface should be improved in order that they may display the blood gas analysis results to the user, relay the testing process status, and also provide device information regarding device health and calibration. A keypad would allow for the user to select settings while either a larger and/or touch activated LCD would allow for user menus to be displayed

8.4 Future Testing and Validation

There are two design requirements that testing with the rice starch substitute could not verify: using whole blood as or sample and to prevent lysing of the blood. The next step in verifying the microfluidic chip and the syringe pump as a fluid movement method would be to perform testing using whole blood samples as opposed to our current rice starch solution. To test for blood lysing, we would perform our Sample Integrity test, Protocol 8.1. We also suggest performing a CCK-8 assay otherwise known as Cell Counting Kit-8 as a cytotoxicity assay [87] in which cells are suspended and incubated with CCK-8 solution. After the incubation period, we measure the absorbance of the CCK-8 which correlates to the number of living cells. By comparing the CCK-8 assays for blood before and after being pulled through the microfluidic cartridge, we can see how many lives cells are left and if any cells were lysed because of the microfluidic channels or the fluid movement method. This would verify if blood lysing occurs in the system.

We also want to make sure blood is not clotting in the channels and obstructing flow. A Factor V assay can be used by measuring the levels of factor V – a protein that aids in enzyme reactions that cause clotting, before and after passing the fluid through the cartridge [88]. This assay would verify that whether or not blood clotting occurs in the cartridge by measuring the percentage of Factor V in the sample. If clotting is a common issue with this system, we also suggest including a heparin, an antithrombin and anticoagulation medication [89]. A small sample of heparin may be placed in the cartridge before use, and when the sample is pulled in the sample would coat the walls of the channel enough to prevent coagulation.

8.5 Heating Elements

For our temperature testing, we placed the microfluidic cartridge on a heating plate. However, a smaller, more efficient heating element dis desired for the final design. The current heating plate requires more power than an Aurdino pin or any microcontroller can give [90]. On the other hand,

portable Heating pads that are small enough to connect to a microcontroller while drawing little power cannot heat up enough in the time frame of 3 minutes [91].

Another solution would be to embed a heating wire or coil into the cartridge material running along the channels. This solution is small and discrete without requiring too much energy. The coil shape and number of coils can be designed to match the desired energy and temperature [92]. However, if the wire is within the cartridge, we would need a way to interface the wire and a power source when the cartridge is inserted into the handheld device. This would also chance the manufacturing process of the bottom plate in order to include the wire within the plate.

8.6 Additional Components

Additional components to consider for the final design include integrating a high grade reusable syringe such as Hamilton® Gastight syringe with a PTFE plunger tip which creates a leak free seal. This syringe series are available in dispensing volumes ranging from 1 μL to 500 μL with high volume resolution, and have a longer lifespan than the current 1 mL plastic syringes currently in use with our model. Our team recommendation is to use a 100 μL Model 1710 SN SYR shown in *Figure 95*. This model in particular allows for custom needle gauge and length to be ordered, while costing $68.00. Additionally, Hamilton can easily custom build a reusable syringe for IL's use should none of their general products fit the device's needs [93].

Figure 95: 100 μL Hamilton Syringe with 22 Gauge Needle

Other component considerations include optimizing the size of the handheld device body and restructuring the device component orientation in order to save space. Additionally, a rechargeable battery would allow the system to be even more portable. With the ideal materials and components, our team strongly believes that the final iteration of this system could easily be the size of a typical smart phone.

9. Conclusion

POC devices allow for the delivery of services and results to patients at the time of care. It opens up the possibilities of better diagnosis due to a reduced wait time for test results while allowing for easy integration with current electronic health records. Not only does this reduce specimen contamination that may occur over travel time, it reduces the potential of potentially mislabeling results patient to patient. Additionally, POC has become the standard for devices used in disaster situations. The shift from lab to hand-held diagnostic device has been apparent and more beneficial to both the patient and healthcare professionals.

In the beginning of this Major Qualifying Project, the team was approached by IL to create a handheld blood gas analyzer utilizing their current sensor technology. This project would not only allow IL to enter the POC market, but also allow us to create a product that rivaled the functionality of competing products and eventually surpass them in cost and versatility. Similar available devices do perform the necessary blood gas panel with a similar analyzer and cartridge design, however at a larger cost per test bracket. Additionally, many of their cartridge designs have a very limited shelf life in addition to requiring refrigeration. It was our intent to design a system with the potential for use beyond the traditional hospital setting such as in field hospitals or at home care in addition to using IL's technology.

Our final device included a syringe pump, disposable cartridge, a microcontroller, and a housing body. Base on the criteria that IL has given to the group, our device satisfies the majority the majority of the needs. The system in its entirety was capable of positioning the sample fluid of rice starch and water over the sensors repeatedly in under 3 minutes with minimal mechanical damage to the sample. The cartridges are capable of holding 100 μL of blood in addition to 100 μL of reagent fluid while the curve channels reduce deformation of the sample granules. Additionally, we have full functionality of the user interface and is capable of warming the fluid to 37°C with the proper heating element. Overall, the housing unit correctly positions the cartridge for interfacing with the syringe pump while holding the micro-controller, syringe, and stepper motor, and peripherals (i.e. LCD screen and LEDs) in place. Furthermore, the device consists entirely RoHS compliant materials that will ensure the safety of the user and sample from heavy metal exposure.

Nevertheless, there are still many considerable improvements necessary to allow the system to be brought to market. Due to the large scope of the project, our team focused on the fluid positioning mechanism of the device. Further investigation into optimizing the device size, sensor implementation, adding a heating element, testing our current system with blood samples, and improving the manufacturing process must be accomplished in order to satisfy the remaining client needs. The project holds much promise and with proper refinement, the device can easily be manufactured to be the size of a cell phone yet will retain the functionality of a benchtop analyzer.

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Appendix A: Testing Protocols

This section lists the testing to be performed in order to ensure quality and accuracy of the disposable cartridge. Below are listed the recommended tests [94, 95]

1.1 Viscosity Testing

Title: Viscosity Testing and Verification Protocol
Author: Nhi Phan, Emily Richardson, Stephany Ruiz, and Tiffany Vo
Version: 1.0 Created 03/03/2017

1. Objective
   The purpose of this protocol is to characterize the viscosity different rice starch to water ratio and to verify its potential use as a blood substitute for preliminary testing purposes.
2. Scope
   A suspension of rice starch in water has been proposed as a potential blood substitute due the viscosity and nature of the rice starch. Different rations will be created using rice starch and water, and be allowed to mix on a stir plate until the time of measuring. A DV2T (Brookfield) viscometer was then calibrated using distilled water and run on the individual test samples, washing the rotational spindle in between tests. This test will determine the viscosity of different starch to water ratios and allow us to select the one closest to the desired viscosity of 3.1 cP.
3. References

4. Applicability
   Cartridge prototype numbers: 3, 4, 5
5. Requirements
   This test is meant to validate the following requirement(s):
   - **The device shall use whole blood.**
6. Equipment
   Analytical Balance
   DV2T Model Viscometer
   Stir Bars
   Stir Plate
7. Materials and Disposables
   Weigh boats
   Rice Starch
   DI Water
   600ml Beakers

8. Test Sample Volume
   8.1 Duration
   i. Each test should last no longer that 2 minutes on the single point averaging setting.
   ii. Total test time should not exceed 2 hours
   8.2 Settings
   i. Set the rotational speed of the spindle to 50 rpm with single point averaging time of 2 minutes
   ii. To save to USB, insert the USB to the back of the unit and then change the pathway on the monitor for saving to USB drive
   iii. Spindle: choose the largest spindle due to its accuracy with lower viscosities (Spindle 18 was found to be optimal for the testing of our materials)
   8.3 Procedure

i. Sample Preparation
    1. Turn on analytical scale
    2. Zero scale with the weight boat
    3. Measure 0.1g of rice starch into the weigh boat

ii. Record weight
    1. Fill 600ml beaker to 500ml mark with DI water
    2. Pour measured rice starch sample into beaker
    3. Place beaker on stir plate and insert clean stir bar
    4. Stir on 300 rev/minute for at least 3 minutes until there are noticeable clumps
    5. Repeat with new beaker and weigh boats for sample sizes of 0.2-0.5 increasing by 0.1g increments. Place all sample mixtures on stir plates until time of testing.

iii. Auto Zeroing
    1. Turn on DV2T module
    2. Ensure the module is level by looking at the bubble in the circular window
    3. Hit the auto zero prompt when it appears on the screen

iv. Control Sample
    1. Ensure settings are appropriate (see part b)
    2. Move unit up rack until the beaker can easily slide under the spindle
    3. Slide 600ml beaker of pure DI water until it is centered under the spindle
    4. Lower unit until the conical region at the top of the spindle is completely submerged. This will calibrate the viscometer to water for later reference.
    5. Press the start Button
    6. Save Data to USB as "Water.Date.Run#", incrementing the # with the number of runs.

v. Sample
    1. Run control sample before start of this procedure
    2. Ensure settings are appropriate
    3. Move unit up rack until the beaker can easily slide under the spindle
    4. Slide 600ml beaker of rice starch mixture until it is centered under the spindle
    5. Lower unit until the conical region at the top of the spindle is completely submerged
    6. Save Data to USB as "grams rice starch.Date.Run#"
    7. Press the start Button
    8. Repeat for all concentrations of rice starch. Run the test 3 times with each sample. Wash the spindle with distilled water between each run

8.4 Data Evaluation

i. Data Collection
    1. Remove USB from DV2T and download results. Load onto computer.
    2. Open the Excel sheet from each run and select the pertinent data.

ii. Sample Viscosity
    1. Find the mean and standard deviation of the total number of runs for each rice starch to water ratio sample.
    2. Create a box and whisker plot in order to evaluate the deviation and consistency of the run for each ratio.
    3. Graph the means of the viscosity for each ratio for each rice mixture on a scatter plot. Using regression, find the line of best fit for the plot.
    4. Use the line of regression to estimate the best ratio of rice starch to water to achieve 3 cP at 50 rpm.

2.1 Seal Testing

Title: Verification Protocol for Microfluidic Cartridge Water Tightness for Fixed Sample Volume
Author: Nhi Phan, Emily Richardson, Stephany Ruiz, and Tiffany Vo
Version: 1.0 Created 2/09/2017

1. Objective
   The purpose of this protocol is to determine the capabilities of our system to maintain a fluid tight seal and not leak any fluid after the aspiration of sample.

2. Scope
   Sample volume testing will be conducted on blood substitute samples (dyed water and rice starch/water mixture) using a 200 μL and 1000 μL pipet to insert and position fluid. This test was conducted on iteration 3 and 4 of our microfluidic cartridge design. We allowed the fluid to sit with the pump not running for 1 minute and 30 seconds. Afterwards, the cartridge will be released from the stand, and the inlet and outlet sides will be sealed off with parafilm. The cartridge will be assessed for leakage points through optical imaging.
3. References

4. Applicability
   Cartridge prototype numbers: 3, 4, 5
5. Requirements
   This test is meant to validate the following requirement(s):

   The device shall limit user exposure to biohazards
   The device shall maintain blood sample integrity

6. Equipment
   Cartridge Stand
   Analytical Balance
   Microscope fitted with camera & timer
7. Materials and Disposables
   Sealed cartridges
   Pipette Tips
   200 uL Pipette
   Distilled Water
   Micro centrifuge tubes
   Micro-milled Microfluidic Cartridge
   Glass Slides
   Food Dye (orange)
   70% ethanol

8. Sample Size Rationale
   In accordance to client need number 1, our device must be capable of withdrawing 100 μL of sample, and thus we will be testing with this sample size.

9. Test Sample Volume
   9.1 Duration
   This test should take no longer that 1.5 hours maximum
   9.2 Set Up
   i. Sample Preparation

1. Obtain two 5 mL micro centrifuge tubes. Weigh the empty tubes on a zeroed scale. Record the weights and label one for rice starch/water and one for water.
2. Obtain distilled water. Measure 4 mL of each liquid into separate 5mL test tubes.
3. Add 100 μL of blue food coloring to the water. Vortex in order to ensure the dye is properly mixed with the water.
4. Weigh the tube of water on the scale. Record measurements for future reference.
5. Weigh the cartridge on the scale and two strips of parafilm. Record this weight.

9.3 Procedure

i. Introduction of Sample to the Cartridge

1. Using the confocal microscope, take images of the cartridge at each sensor point. Save for comparison
2. Measure 100 μL of sample from the microcentrifuge tube using the 200 μL pipet. Weight the microcentrifuge tube on the scale and record its weight after removing this volume.
3. Place the tip of the pipet on the inlet of the cartridge and allow the fluid to be pulled through. Use the 1000 μL to apply enough pressure to pass the fluid and let it position over the sensor holes.
4. Let the cartridge sit for five minutes. Afterwards, optically inspect the cartridge for areas of leakage and note.
5. Using the confocal microscope, take images of the channel sensors allowing clearance on both sides to be seen. Save all such images.
6. To remove the cartridge, unscrew the cartridge from the platform. Parafilm both the inlet and outlet of the cartridge to prevent spilling.
7. Weigh the cartridge on the scale afterwards and record weigh.
8. Once the procedure has been run with dyed water, remove the cartridge and flush with distilled water at least 4 times or until no dye is observed in the cartridge. Allow the cartridge to fully dry and use a vacuum to remove any remaining liquid. May also use tape to remove any particulates that may have settled on the cartridge.
9. Repeat procedure with rice starch mixture.

10. Data Evaluation

10.1 Data Collection

1. Open the confocal microscope imaging software, ZEN on your screen.
2. Open the image of the first sensor before fluid was introduced into the cartridge. Measure the RGB values of the middle most pixels on either side of the channel. Record for future reference.
3. Open the image of the 1 sensor with fluid introduced into the channel. Again, measure the RGB values of the middle most pixels on either side of the channel. Record for future reference.
4. Repeat until all nine sensors have had their RGB values recorded before and after fluid has been introduced.

10.2 Determination of leakage

1. Take the difference of the RGB values using this formula:

   i. $RGB(without\ fluid) - RGB(withfluid) = Difference$

2. Do so for each pair of sensor images.
3. Mark any differences greater than 30. These differences will be labeled as leakage.
4. Additionally note any areas of large leakage areas observed during testing.

3.1 Dynamic Flow Testing

Title: Verification Protocol for Microfluidic Cartridge Dynamic Fluid Flow Rate for Fixed Sample Volume
Author: Nhi Phan, Emily Richardson, Stephany Ruiz, and Tiffany Vo
Version: 1.0 Created 1/26/2017

1. Objective:
   The purpose of this protocol is to determine the rate of flow of 100 μL of fluid through acrylic microfluidic cartridges at various settings of the stepper motor. This will be done by measuring the time it takes to aspirate 100 μL of blood through a known volume of microfluidic channel.
2. Scope:
   Sample volume testing will be conducted on blood substitute samples (water & canola oil) using the construed stepper motor at different rotational speeds. This test was conducted on iteration 3 and 4 of our microfluidic cartridge design. The speeds chosen for the stepper motor were…..
   - Setting 1 - 30 μL/sec
   - Setting 2 - 40 μL/sec
   - Setting 3 - 50 μL/sec
3. References:
   L0024016214 GEM 5000 Sample Volume Protocol Rev 03
4. Applicability:
   Cartridge prototype numbers: 3, 4, 5
5. Requirements:
   This test is meant to validate the following requirement(s):
   - **The device shall position the patient blood sample repeatedly over blood sensors.**
   - The device shall complete analysis in less than 3 minutes.
6. Equipment:
   MQP Stepper Motor
   Harvard Apparatus Infusion/Withdrawal Syringe Pump
   Cartridge Stand
   Analytical Balance
   Camera with timer
7. Materials and Disposables:
   Pipette Tips
   1000 μL Pipette
   Canola Oil
   Distilled Water
   Micro centrifuge tubes
   Micro-milled Microfluidic Cartridge
   Glass Slides
   Blue Food Coloring
   Yellow Food Coloring
   10% ethanol
8. Sample Size Rationale
   In accordance to client statement, our device must be capable of withdrawing 100 μL of sample.
9. Test Sample Volume
   9.1 Duration

Test should take up to 2 hours including set up.

9.2 Setup

i. Instrument Setup

A. For testing with Harvard Apparatus Infusion/Withdrawal Syringe Pump

1. Begin by first placing the 1 mL syringe with filter and capped needle on the syringe pump, allowing the capped needle to protrude enough that it may interface with the cartridge later on.
2. Attach plate that latches onto the notch of the syringe plunger.
3. Start the pump and input the desired volumetric flow speed

B. For Testing with the Stepper Motor

1. Connect a computer to the Arduino board, stepper motor driver, and stepper motor.
2. Connect syringe to stepper motor station.
3. Run system program in the Arduino software. Stand by for further instructions.

C. Mark in sharpie the two position points from which we will measure the distance of fluid flow over for easier viewing.

D. Set up camera above stage to record fluid movement over cartridge.

ii. 9.2.2 Sample Preparation

1. Obtain two 5 mL micro centrifuge tubes. Weigh the empty tubes on a zeroed scale. Record the weights and label one for oil and one for water.
2. Obtain canola oil and distilled water. Measure 4 mL of each liquid into separate 5mL test tubes.
3. Add 100 μL of blue food coloring to the canola oil. Added 100 μL of yellow food coloring to the distilled water. Mix both so that the dye is distributed evenly throughout the liquid.
4. Weigh each tube of oil and water on the scale. Record measurements for future reference.

9.3 Procedure

i. Cartridge Installation and Stepper Motor Warm Up

1. Place cartridge on the cartridge stand. Slide cartridge on stand until the syringe tip from the syringe pump lines up properly with the backend inlet of the cartridge.
2. Screw the wing nut down until the cartridge is secure in the stand and does not move.
3. To ensure secureness, double check the following:
   a. Make sure the syringe tip does not slide out of the inlet.
   b. Make sure the cartridge does not move.

ii. Introduction of Sample to the Cartridge

1. Once motor is running, measure 100 μL of oil from the microcentrifuge tube using the pipet. Weight the microcentrifuge tube on the scale and record its weight after removing this volume.
2. Start camera to record fluid flow. Place the tip on the inlet of the cartridge and allow the oil to be pulled through. After fluid has passed over the distance, stop the stepper motor and allow the fluid to settle.
3. Purge cartridge of fluid using distilled water. Dry cartridge using a vacuum. Analyze channels in microscope to check for residual fluid.
4. Repeat five times with the remaining sample. Remember to measure the mass of the centrifuge tube every time before and after extracting sample.
5. This procedure is to be repeated with different stepper motor speed.

10. Data Evaluation

10.1 QC and Proficiency Sample Density

1. Calculate the mean mass of the five trials and calculate the density using the equation below. Density = (mean sample mass)/ Volume (100 μL)
2. Compare density to NIST standard

10.2 Sample Volume

1. Use the following equation to calculate the

Volume = (Mass1-Mass2)/Density of Oil

2. Find P value.

10.3 Data Collection

1. Remove SD card from camera and download videos of each trial run onto the computer.
2. Find the start time and end time of each video when fluid reaches point 1 and point 2. Find the difference in the two time points to find time overlapped to defined distance.
3. Use time overlapped as well as known volume of the section of channel to calculate fluid flow rate. Find this value for each video and calculate the standard deviation and mean for each trial.

4.1 Stationary Fluid Positioning

Title: Verification Protocol for Microfluidic Cartridge Stationary Positioning for Fixed Sample Volume
Author: Nhi Phan, Emily Richardson, Stephany Ruiz, and Tiffany Vo
Version: 1.0 Created 2/09/2017

1. Objective

   The purpose of this protocol is to determine the capabilities of our system to position the fluid sample of 100 μL at the sensors and maintaining fluid position at this point for at least one minute. This will be done by recording fluid placement over the sensors, and then monitoring fluid movement.

2. Scope

   Sample volume testing will be conducted on blood substitute samples (dyed water and rice starch/water fluid) using the construed stepper motor at different rotational speeds. This test was conducted on iteration 3 and 4 of our microfluidic cartridge design. The speed chosen for the stepper motor was 30 μL/sec to place fluid over the sensors. We allowed the fluid to sit with the pump not running for 1 minute and 30 seconds. At the conclusion, the cartridge was assessed for leakage under a microscope.

3. References

4. Applicability

   Cartridge prototype numbers: 4+, with syringe inlet

5. Requirements

   This test is meant to validate the following requirement(s):

   The device shall position the patient blood sample repeatedly over blood sensors.

   The device shall operate in any handheld orientation.

6. Equipment

   Stepper Motor
   Cartridge Stand
   Analytical Balance
   Microscope fitted with camera & timer

7. Materials and Disposables

   1000 μL pipette with tips
   Distilled Water
   Food coloring - blue, yellow
   10% ethanol
   Microcentrifuge tubes
   Micro-milled Microfluidic Cartridge
   Glass Slides

8. Sample Size Rationale

   In accordance to our client's requirement, our device must be capable of withdrawing no more than 100 μL of sample, and thus we will be testing with this sample size.

9. Test Sample Volume Procedure

   9.1 Duration

   This test should take no longer that 1.5 hours maximum

   9.2 Set Up

   i. Instrument Setup

      1. Connect a computer to the arduino board, stepper motor driver, and stepper motor.
      2. Connect syringe to stepper motor station.
      3. Run system program in the Arduino software. Stand by for further instructions.

   ii. Sample Preparation

1. Weigh two empty 5 mL microcentrifuge tubes on a zeroed scale. Record the weights and label one for rice starch/water and one for water.
2. Obtain distilled water. Measure 4 mL of liquid into a 5mL test tubes.
3. Add 100 μL of blue food coloring to the canola oil. Added 100 μL of yellow food coloring to the distilled water. Mix both so that the dye is distributed evenly throughout the liquid.
4. Weigh each tube of oil and water on the scale. Record measurements for future reference.
5. Weigh the cartridge on the scale and two parafilm strips. Record this weight.

9.3 Test Procedure

a. Cartridge Installation and Stepper Motor Warm Up

1. Place cartridge on the cartridge stand. Slide cartridge on stand until the syringe tip from the syringe pump lines up properly with the backend inlet of the cartridge.
2. Screw the wing nut down until the cartridge is secure in the stand and does not move.
3. To ensure secureness, double check the following:
    - Make sure the syringe tip does not slide out of the inlet.
    - Make sure the cartridge does not move.

9.4 Introduction of Sample to the Cartridge

1. Measure 100 μL of sample from the microcentrifuge tube using the 200 μL pipet. Weight the microcentrifuge tube on the scale and record its weight after removing this volume.
2. Start camera to record fluid flow. Place the tip on the inlet of the cartridge. Initiate the stepper motor to run. Allow the fluid to be pulled through.
3. After fluid has passed over the distance, make sure the stepper motor stops. Allow the fluid to settle.
4. Record with the camera for 90 seconds the cartridge to test for fluid flow.
5. Unscrew the wing nuts, slide cartridge away from syringe pump and remove from the platform. Parafilm both the inlet and outlet of the cartridge to prevent spilling.
6. Weigh the cartridge on the scale afterwards and record weigh.
7. Repeat procedure 9.c nine more times with the remaining sample of water. Remember to measure the mass of the centrifuge tube every time before and after extracting sample.
8. Once the procedure has been run with dyed water, unclasp and wash the cartridge using water and soap using a soft sponge. Perform a second wash with 10% ethanol. Allow the cartridge to fully dry and use a vacuum to remove any remaining liquid. May also use tape to remove any particulates that may have settled on the cartridge.

9.5 Testing notes:

1. Repeat procedure with rice starch mixture.
2. Perform the same test in several cartridge positions to ensure that the procedure and stationary flow can perform in different orientations.

10. Data Evaluation

10.1 Sample Volume

1. Compare weight of cartridge before and after to see if there was any fluid loss.

10.2 Data Collection

1. Remove SD card from camera and download videos of each trial run onto the computer.
2. Find the start time and end time of each video when fluid reaches Point 1 and Point 2. Find the difference in the two time points to find time overlapped to defined distance. Verify that the fluid did reach and cover the sensors.
3. Select the first frame and the final frame of the 90 second recording.
4. Visually determine areas of fluid movement when the pump was off. Measure using an image analysis program such as ZEN or ImageJ and verify using pixel values. Mark for future improvements.

5.1 Angle Fluid Containment

Title: Verification Protocol for Microfluidic Cartridge Fluid Containment for Varying Handheld Angles
Author: Nhi Phan, Emily Richardson, Stephany Ruiz, and Tiffany Vo
Version: 1.0 Created: 03/02/2017

1. Objective
   The purpose of this protocol is to determine the capabilities of our system to maintain sample alignment a varying handheld angles within the device and outside of it. It is also to verify fluid containment if the seal is broken after testing
2. Scope
   Fluid containment testing will be performed with a total of 200ul of blue dyed water.
3. References

4. Applicability
   Cartridge prototype numbers: 3, 4, 5
5. Requirements
   This test is meant to validate the following requirement(s):
   - **The device shall operate at any handheld position**
6. Equipment
   Angling Cartridge Stand
   Analytical Balance
   Protractor
   Stop Watch
   Video Camera
7. Materials and Disposables
   Pipette Tips
   1000 µL Pipette
   Distilled Water
   Micro-milled Microfluidic Cartridge
   Glass Slides
   50ml beaker
   Blue Food Coloring
   Yellow Food Coloring
   10% ethanol
8. Sample Size Rationale
   In accordance to client need number 1, our device must be capable of withdrawing 100 µL of sample, and thus we will be testing with this sample size.
9. Test Sample Volume
   9.1 Duration
   This test should take no longer that 1.5 hours maximum
   9.2 Set Up
   i. Sample Preparation
      1. Obtain a clean 50ml beaker
      2. Added 500 µL of blue food coloring to the distilled water. Mix both so that the dye is distributed evenly throughout the liquid.
   9.3 Procedure
   i. Introduction of Sample to the Cartridge
      1. Place cartridge in adjustable stand and secure with clamps. Begin with cartridge outlet at an 0˚ angle
      2. Measure 100 µL of sample from the 50ml beaker using the 200 µL pipet.

3. Introduce 100ul of fluid into the cartridge with the pipet. Insert air in after the fluid until fluid is resting on sensor holes.
4. Insert second 100ul into the cartridge with pipet, again inserting air until this fluid is resting over the sensors.
5. Mark with an erasable pen the top and bottom limits of the fluid

ii. Monitoring of fluid alignment

1. Start camera to record fluid movement.
2. Cap the inlet with a rubber stopper
3. Insert the syringe into the outlet
4. Record cartridge at this 0° angle for 2 minutes. Ensure no fluid leakage or spilling.
5. Repeat 2 minute observation with angle at 30°, 45°, 60°, and 90°

iii. Monitoring of fluid containment

1. Return cartridge to 0° position with original fluid alignment (if necessary air blow out current fluid and refill and mark fluid position)
2. Record cartridge for 30 seconds while the syringe is removed from the outlet
3. Record for another 30 seconds while the rubber stopper is removed from the inlet
4. Repeat procedure with observations at 30°, 45°, 60°, and 90°

10 Data Evaluation

10.1Data Collection

1. Remove SD card from camera and download videos of each trial run on to the computer.
2. Note the discrepancies of starting and ending positions of fluid at each angle
3. Note if there is gradual movement throughout the 2 minutes at each angle
4. Select the first frame and the final frame of each angle's recording and overlay them to measure the change in height of the fluid
5. Measure using ImageJ and verify using pixel values. Mark for future improvements

Potential elaboration of this protocol – perform positioning test in several cartridge positions.

6.1 User Interface Verification

Title: Providing LED Feedback Based on User and Sensor Inputs
Author: Nhi Phan, Emily Richardson, Stephany Ruiz, and Tiffany Vo
Version: 1.0 Created 02/15/2017

1. Objective
   The purpose of this protocol is to determine the ability of the electronic platform to provide user feedback in the form of LEDs based on user and sensor inputs. Three LEDs will be connected to sensors in the disposable cartridge and a button as input sources and each will light up or blink based on the current status of the system.
2. Scope
   There are going to be three LEDs to represent the status of the system. The LED1 will light to show the system is ready to begin and will blink after being signaled to start by the user. Once the first position sensor is reached, LED1 will turn off and LED2 will start blinking. After the data collection is done, LED2 will turn off and LED3 will turn on to show the reading is ready. The system will reset when prompted by the user.
3. References

4. Applicability
   Any cartridge prototype with sensors
5. Requirements
   This test is meant to validate the following requirement(s):
   The disposable component shall interface with the device electronics to support sensor function.
   The device conveys current test status to user.
   The device leverages open source electronic platforms.
6. Equipment
   Arduino RedBoard
   Stepper motor driver board
   Stepper motor
   3 LEDs - red, yellow, green
   3 330Ω resistors
   Optical sensors
   Position sensors

7. Materials and Disposables
   1. Opaque fluid
8. Procedure
   1. Set Up
      1. Create a test fluid that is opaque
   2. Procedure
      1. Before you begin, LED1 is on.
      2. Press button to move fluid and LED1 starts blinking.
      3. When position sensor is triggered, LED1 turns off.
      4. Sensors start collecting data, LED2 is blinking.
      5. When reading is done, LED2 turns off, LED3 turns on.
9. Evaluation
   Make sure that the proper LED is performing the correct instructions for changing system statuses.

7.1 Temperature Control Verification

Title: Verification Protocol for Maintaining Temperature
Author: Nhi Phan, Emily Richardson, Stephany Ruiz, and Tiffany Vo
Version: 1.0 Created 3/4/2017

1. Objective
   This protocol is to make sure that the cartridge is able to maintain its temperature in the range of 37℃ to be able to perform sensor analysis at body temperature.
2. Scope
   The cartridge will be places on a heating plate to 37℃ and then the sample will be drawn through. A wire thermometer/temperature sensor will be placed into the channel. The cartridge will sit for 1 minute, the time it takes for the sensors to read the data. The thermometer will monitor the temperature over the course of that minute to record any temperature changes during the analysis time period.

References

4. Applicability
   Cartridge prototype - temperature channels
5. Requirements
   This test is meant to validate the following requirement(s):
   The device shall perform analysis at body temperature (37.4 +/- 0.3 C).
6. Equipment
   Heating plate
   Wire thermometer
7. Materials and Disposables
   Micro-milled Microfluidic Cartridge with thinner channels
   Distilled water
   Pipette
8. Procedure
   1. Place microfluidic cartridge onto heating plate. Heat until the temperature is 37℃.
   2. Draw 100 μL of distilled water into the cartridge using a pipette.
   3. Slide thermometer wire into the cartridge until it reaches the sample water.
   4. Record the temperature reads for 1 minute.
   5. Repeat procedure 9 times to validate temperature readings.
9. Data Analysis
   - Graph and analyze the temperature readings for each test.
   - Determine if the temperature change over the 1 minute is too drastic or if it remains in the 37℃ temperature range.

8.1 Turbulence and Contamination Control

Title: Verification Protocol for Maintaining Sample Integrity
Author: Nhi Phan, Emily Richardson, Stephany Ruiz, and Tiffany Vo
Version: 1.0 Created 2/09/2017

1. Objective
   This protocol determines if the cartridge, syringe pump, and fluid flow will harm the integrity of the sample. There should be no turbulent flow in the channels, which can cause blood lysing. There should be no air and reagent contamination either. To run this test, we will visually check the fluid sample before and after moving fluid through the system for any particle size changes or the presence of foreign substances.
2. Scope
   A sample using rice starch as a blood cell substitute will be made. It will be placed into a hemocytometer and viewed under a microscope for image analysis of the particles. The sample will then run through the cartridge and syringe pump system. Some of the sample will be extracted from the cartridge and analyzed under a microscope with a hemocytometer as before. Look for any changes in size of particles or the presence of foreign substances. Note any important observances or changes in sample integrity.
3. References

4. Applicability
   Cartridge prototype numbers: 4+
5. Requirements
   This test is meant to validate the following requirement(s):
   The device shall maintain blood sample integrity (eliminate contamination air and reagent, reduce hemolysis).
   The device shall complete analysis on whole blood.
6. Equipment
   Stepper motor
   Cartridge stand
   Microscope
   Analytical balance
7. Materials and Disposables
   Micro-milled Microfluidic Cartridge
   Hemocytometer
   Pipettes and tips
   Distilled water
   Rice starch
8. Procedure
   8.1 Duration
   This test should take no longer that 2 hours maximum.
   8.2 Set Up
   i. Instrument Setup
      1. Connect a computer to the Arduino board, stepper motor driver, and stepper motor.
      2. Connect syringe to stepper motor station.
      3. Run system program in the Arduino software. Stand by for further instructions.
   ii. Sample Preparation

1. Mix 1g of rice starch for every 1 mL of distilled water.
2. Mix to ensure rice starch integrates with water well.

iii. ImageJ Calibration

1. In ImageJ draw a line along the edge of the hemocytometer grid. Go to the "Analyze" menu and select "Set Scale".
2. Input the known distance, the length of grid in μm and pixel aspect ratio as 1.
3. Select "Global" before hitting Ok.

8.3 Test Procedure

*At each microscope stage, be sure to record the image.

i. Cartridge Installation and Stepper Motor Warm Up

1. Place cartridge on the cartridge stand. Slide cartridge on stand until the syringe tip from the syringe pump lines up properly with the backend inlet of the cartridge.
2. Screw the wing nut down until the cartridge is secure in the stand and does not move.
3. To ensure secureness, double check the following:
    a. Make sure the syringe tip does not slide out of the inlet.
    b. Make sure the cartridge does not move.

ii. Analysis of Sample Before

1. Measure 10μL of the sample and place into the hemocytometer.
2. Place hemocytometer under the microscope and calibrate ImageJ as described in "Set Up."
3. Measure the length of 10 different rice starch clumps and find the average size.

iii. Run Sample through the Cartridge

1. Extract sample into a syringe. Place the tip on the front inlet of the cartridge. Initiate the stepper motor to run. Allow 100 μL of fluid to be pulled through the cartridge.
2. Remove sample syringe from the inlet when stepper motor completes fluid flow.

iv. Analysis of Sample After

1. Extract 10 μL of the sample from the cartridge with a pipette and place into another hemocytometer chamber.
2. Measure the length of 10 different rice starch clumps and find the average size.
3. Count the number of any foreign particles found in the sample.

v. Clean the cartridge.

vi. Repeat procedure 9 times to validate any changes and average values.

8.4 Data Analysis

1 Compare the average rice starch particle lengths before and after being pulled through the cartridge.

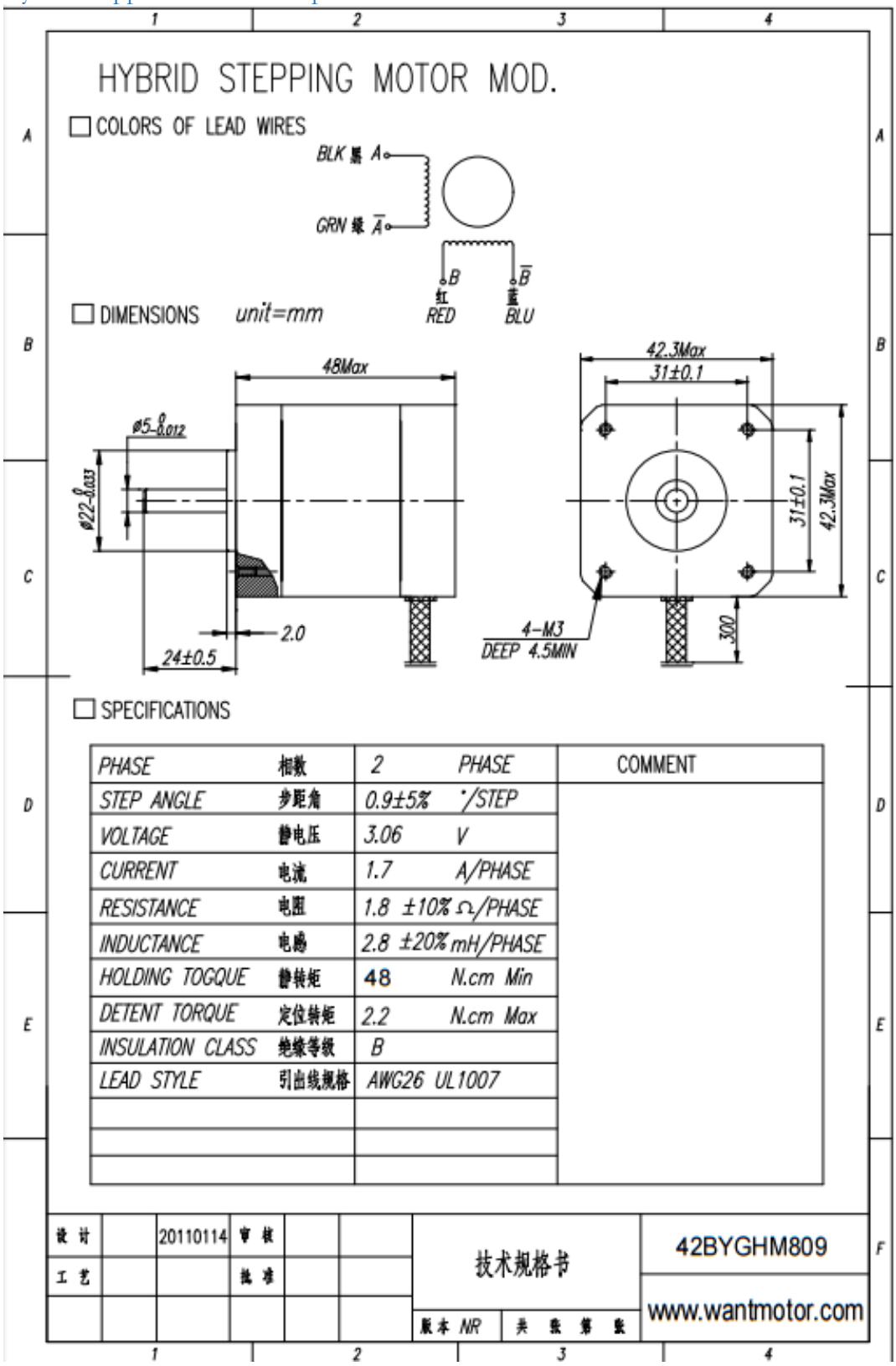
2 Determine if there was a decrease in rice particle size, which correlates to hemolysis from turbulence through the system.

3 Determine if there was a significant increase in foreign particles, which correlates to the system allowing for air and reagent contamination.

4 Measure the statistical standard deviation and determine the significance to maintaining the sample integrity.

Appendix B: Data Sheets

1.1 Hybrid Stepper Motor from SparkFun



PHASE	相数	2	PHASE	COMMENT
STEP ANGLE	步距角	0.9±5%	°/STEP	
VOLTAGE	静电压	3.06	V	
CURRENT	电流	1.7	A/PHASE	
RESISTANCE	电阻	1.8 ±10%	Ω/PHASE	
INDUCTANCE	电感	2.8 ±20%	mH/PHASE	
HOLDING TOGQUE	静转矩	48	N.cm Min	
DETENT TORQUE	定位转矩	2.2	N.cm Max	
INSULATION CLASS	绝缘等级	B		
LEAD STYLE	引出线规格	AWG26 UL1007		

设 计		20110114	审 核			技术规格书	42BYGHM809
工 艺			批 准				
						版本 NR 共 张 第 张	www.wantmotor.com

Appendix C: Stepper Motor Code

```
//Declare pin functions on Arduino
#define stp 2
#define dir 3
#define MS1 4
#define MS2 5
#define MS3 6
#define EN  7

//Declare variables for functions
char user_input;
int x, y, state;

void setup() {
  pinMode(stp, OUTPUT);
  pinMode(dir, OUTPUT);
  pinMode(MS1, OUTPUT);
  pinMode(MS2, OUTPUT);
  pinMode(MS3, OUTPUT);
  pinMode(EN, OUTPUT);
  pinMode(12, OUTPUT);
  pinMode(11, OUTPUT);
  pinMode(10, OUTPUT);
  resetBEDPins(); //Set step, direction, microstep and enable pins to default states
  resetLEDs();
  Serial.begin(9600); //Open Serial connection for debugging
  introScreen();
}

//Main loop
void loop() {
  while(Serial.available()){
     user_input = Serial.read(); //Read user input and trigger appropriate function
     digitalWrite(EN, LOW); //Pull enable pin low to set FETs active and allow motor control

      if (user_input =='1') {
        sixteenthModeForward();
      }
      else if(user_input =='2'){
        sixteenthModeReverse();
      }
      resetBEDPins();
      if(user_input =='3'){
        digitalWrite(12, LOW); // turn off LED1
        LED2blink();
        digitalWrite(10, HIGH); // turn on LED3
        Serial.println("To reset, input x:");
```

```
      }
      if(user_input == 'x') {
        resetBEDPins();
        introScreen();
        resetLEDs();
      }
  }
}

void introScreen(){
  Serial.println("Begin motor control");
  Serial.println();
  //Print function list for user selection
  Serial.println("Enter number for control option:");
  Serial.println("1. Step forward at 1/16 microsteps.");
  Serial.println("2. Step reverse at 1/16 microsteps.");
  Serial.println("3. 'Read Sensors'/Blink LED2.");
  Serial.println();
}

//Reset Big Easy Driver pins to default states
void resetBEDPins(){
  digitalWrite(stp, LOW);
  digitalWrite(dir, LOW);
  digitalWrite(MS1, LOW);
  digitalWrite(MS2, LOW);
  digitalWrite(MS3, LOW);
  digitalWrite(EN, HIGH);
}

void resetLEDs(){
  digitalWrite(12, HIGH);
  digitalWrite(11, LOW);
  digitalWrite(10, LOW);
}

void LED2blink() {
  for (int i=0; i<7; i++) {
    digitalWrite(11, HIGH);    // Turn on the LED
    delay(500);
    digitalWrite(11, LOW);    // Turn off the LED
    delay(500);
  }

  Serial.println("Completed Sensor Reading");
  Serial.println();
}
```

```
// 1/16th microstep foward mode function
void sixteenthModeForward(){
  digitalWrite(dir, LOW); //Pull direction pin low to move "forward"
  digitalWrite(MS1, HIGH); //Pull MS1,MS2, and MS3 high to set logic to 1/16th microstep
resolution
  digitalWrite(MS2, HIGH);
  digitalWrite(MS3, HIGH);
  //44800
  for(x= 1; x<2000; x++){  //Loop the forward stepping enough times for motion to be visible
    if (x%50 == 0){
      digitalWrite(12, HIGH); //turn on LED1
    }
    digitalWrite(stp,HIGH); //Trigger one step forward
    delay(1);
    if (x%100 == 0){
      digitalWrite(12, LOW); //Turn off LED1
    }
    digitalWrite(stp,LOW); //Pull step pin low so it can be triggered again
    delay(1);
  }
  digitalWrite(12, LOW);
  Serial.println("Completed Infusion");
  Serial.println();
}

// 1/16th microstep reverse mode function
void sixteenthModeReverse(){
  digitalWrite(dir, HIGH); //Pull direction pin high to move "reverse"
  digitalWrite(MS1, HIGH); //Pull MS1,MS2, and MS3 high to set logic to 1/16th microstep
resolution
  digitalWrite(MS2, HIGH);
  digitalWrite(MS3, HIGH);
  // 44800
  for(x= 1; x<2000; x++){  //Loop the forward stepping enough times for motion to be visible
    if (x%50 == 0){
      digitalWrite(12, HIGH); //turn on LED1
    }
    digitalWrite(stp,HIGH); //Trigger one step forward
    delay(1);
    if (x%100 == 0){
      digitalWrite(12, LOW); //Turn off LED1
    }
    digitalWrite(stp,LOW); //Pull step pin low so it can be triggered again
    delay(1);
  }
  digitalWrite(12, LOW); //Turn off LED1
  Serial.println("Completed Withdraw");
  Serial.println();
```

```
}

// 1/8th microstep reverse mode function
void eighthModeReverse(){
  Serial.println("Stepping at 1/8th microstep mode.");
  digitalWrite(dir, HIGH); //Pull direction pin high to move "reverse"
  digitalWrite(MS1, HIGH); //Pull MS1,MS2, and MS3 high to set logic to 1/16th microstep
resolution
  digitalWrite(MS2, HIGH);
  digitalWrite(MS3, LOW);
  //22400
  for(x= 1; x<3000; x++){  //Loop the forward stepping enough times for motion to be visible
    if (x%50 == 0){
      digitalWrite(12, HIGH); //turn on LED1
    }
    digitalWrite(stp,HIGH); //Trigger one step forward
    delay(1);
    if (x%100 == 0){
      digitalWrite(12, LOW); //Turn off LED1
    }
    digitalWrite(stp,LOW); //Pull step pin low so it can be triggered again
    delay(1);
  }
  Serial.println("Completed Withdraw");
  Serial.println();
}

// 1/8th microstep foward mode function
void eighthModeFoward(){
  Serial.println("Stepping at 1/8th microstep mode.");
  digitalWrite(dir, LOW); //Pull direction pin low to move "forward"
  digitalWrite(MS1, HIGH); //Pull MS1,MS2, and MS3 high to set logic to 1/16th microstep
resolution
  digitalWrite(MS2, HIGH);
  digitalWrite(MS3, LOW);
  //22400
  for(x= 1; x<3000; x++){  //Loop the forward stepping enough times for motion to be visible
    if (x%50 == 0){
      digitalWrite(12, HIGH); //turn on LED1
    }
    digitalWrite(stp,HIGH); //Trigger one step forward
    delay(1);
    if (x%100 == 0){
      digitalWrite(12, LOW); //Turn off LED1
    }
    digitalWrite(stp,LOW); //Pull step pin low so it can be triggered again
    delay(1);
  }
```

```
  Serial.println("Completed Infusion");
  Serial.println();
}
```

Appendix D: Cartridge Design Iterations

This section contains all the design iterations of our cartridge designs. All models were created in SOILDWORKS and were saved as .SLDPRT files.

1.1 Iteration 1: Differential Pressure Design

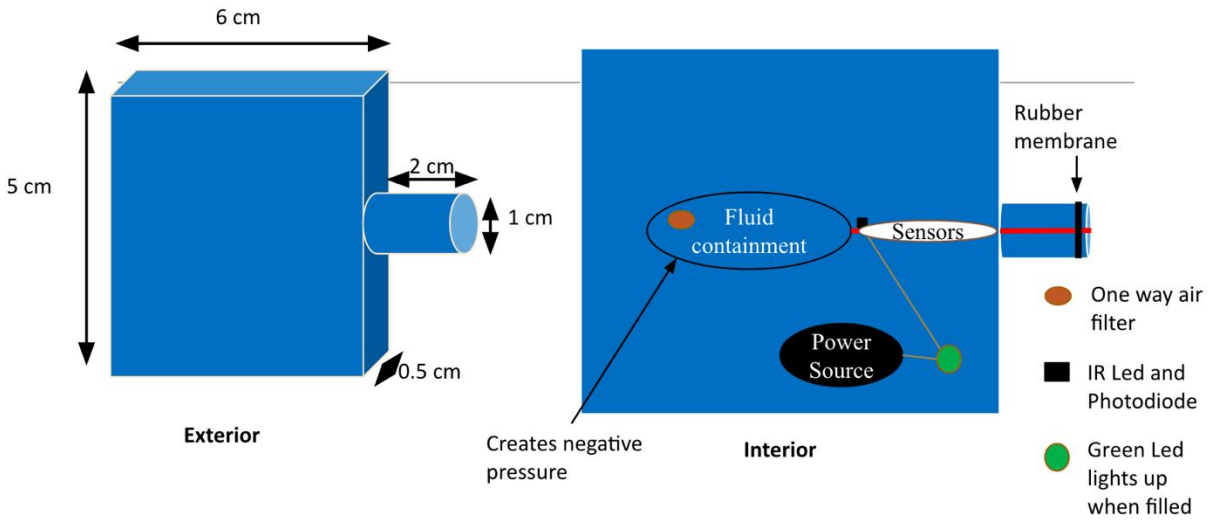


Figure 96: Differential Pressure Cartridge Design Schematic

1.2 Iteration 2: Pressure Design with Reservoir

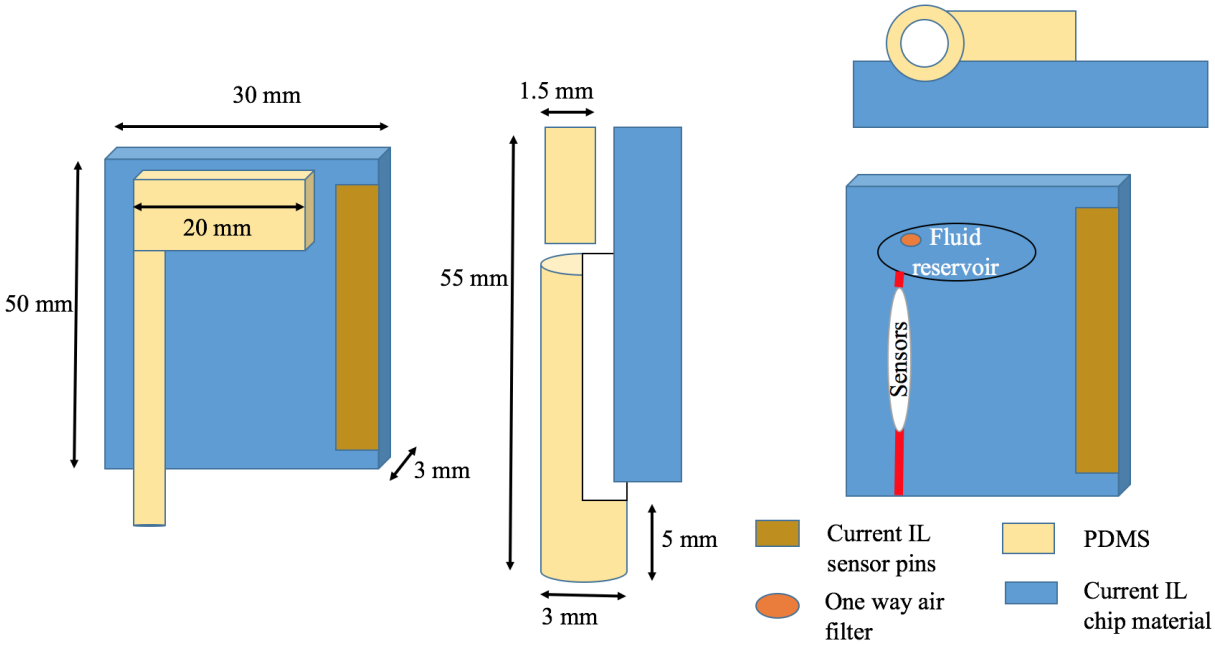


Figure 97: Pressure Cartridge Design with Fluid Reservoir

1.3 Iteration 3: Peristaltic Pump Design

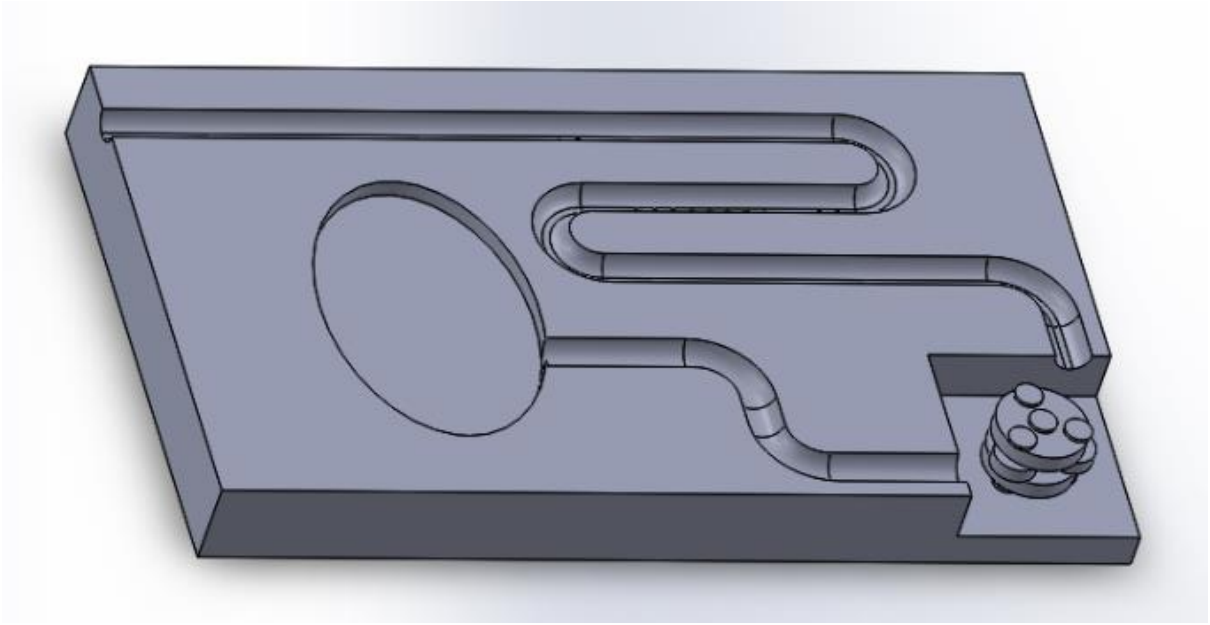


Figure 98: Peristaltic Pump Cartridge Design without Top Plate and Silicone Tubing

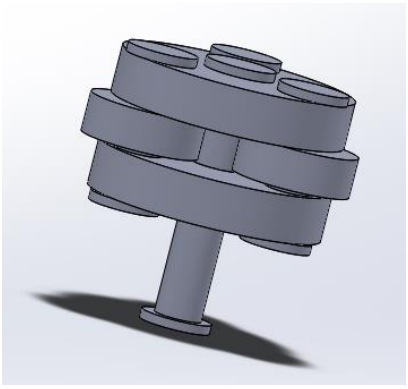


Figure 100: Side View of Peristaltic Pump Rotor with Axel

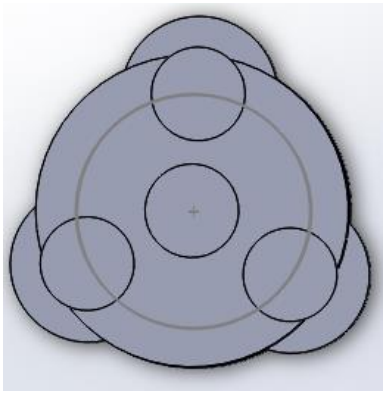


Figure 99: Top View of Peristaltic Pump Rotor with Axel

1.4 Iteration 4: Syringe Pump Design with Base Groves

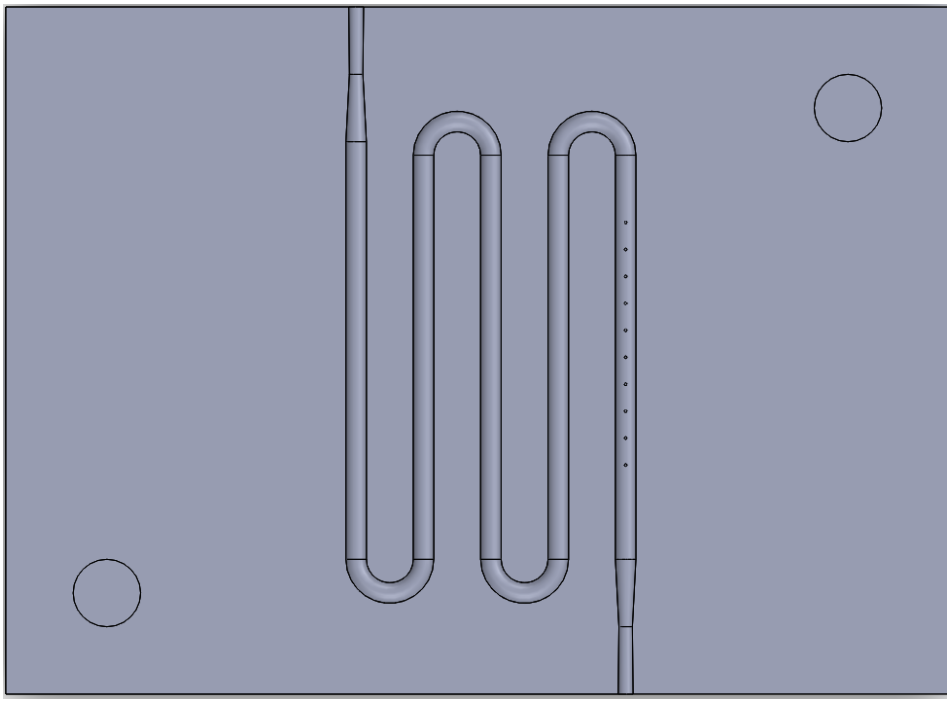


Figure 101: Top Plate with sensor holes and 22 gauge need inlet and outlet

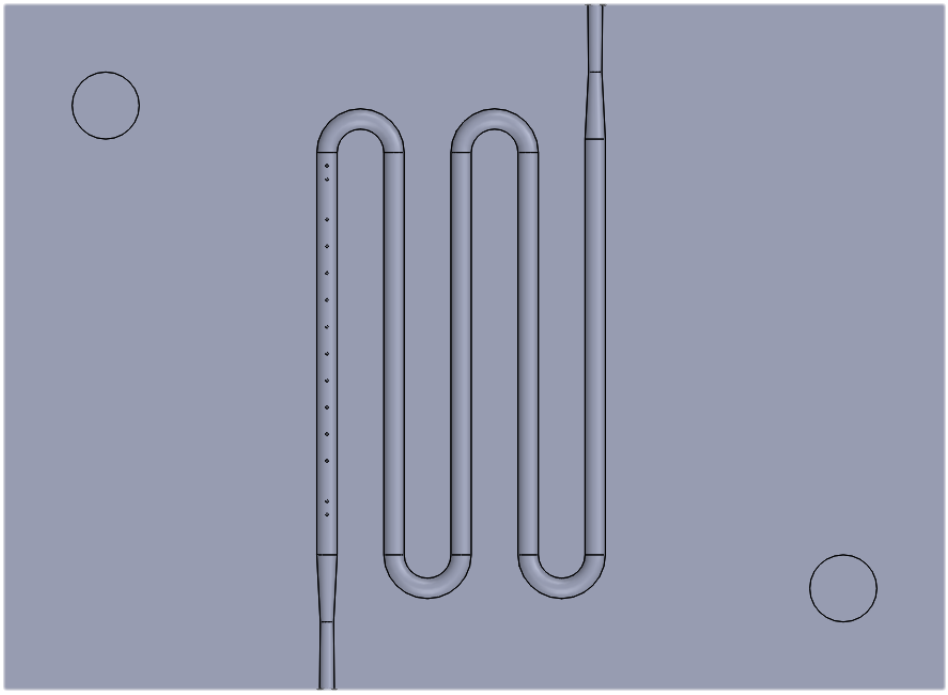


Figure 102: Bottom Plate with sensor holes and 22 gauge needle inlet and outlet

1.5 Iteration 5: Syringe Pump Design with Tongue in Groove Channels

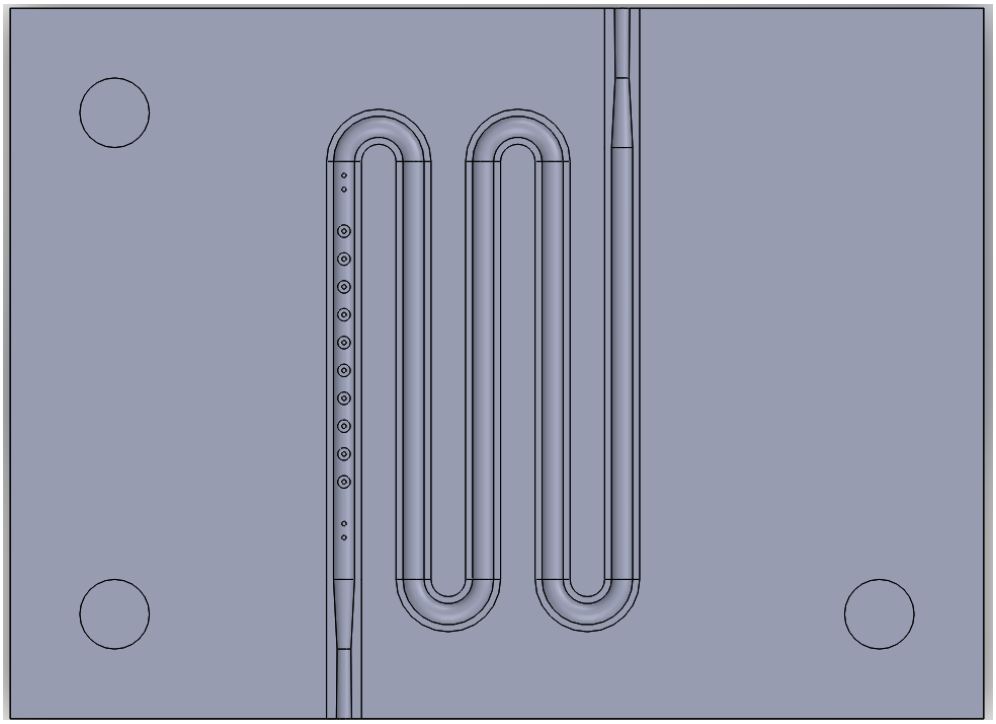


Figure 103: Top down view of bottom plate displaying the sensor positioning holes

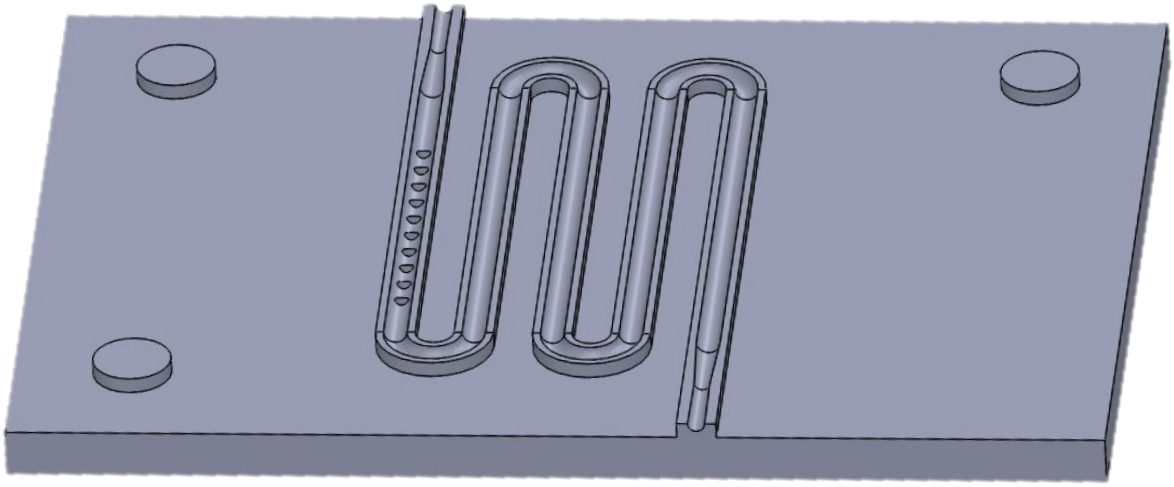


Figure 104: Top plate of the cartridge

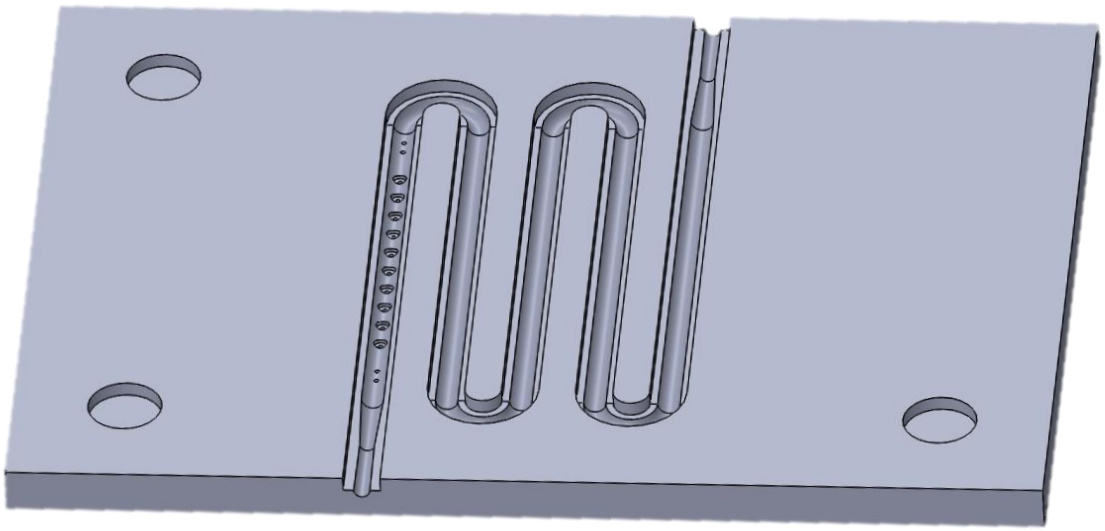


Figure 105: Bottom pate of the cartridge

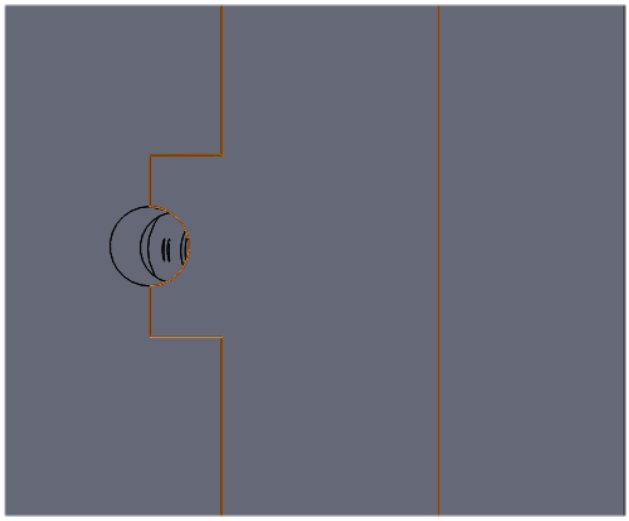


Figure 106: Profile view of interlocking channels when two plates are adhered together