Spring 2015

NASA Limited Inflight Lab Sensor

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Honors Research Project

Submitted to

The Honors College

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Major: Biomedical Engineering
Project Sponsor: Dr. DeVon Griffin
Number of Project Credits: 2

NASA Limited Inflight Lab Sensor

The National Aeronautics and Space Administration (NASA) is currently developing the capabilities needed to send humans to Mars in 2030 for a three year mission. Astronaut health must be monitored monthly during such a lengthy mission. The UA Enterprise Senior Design Team worked to design a reusable, novel blood panel cartridge system to monitor astronaut health and to minimize waste production. Most current cartridges are composed of polydimethylsiloxane (PDMS). PDMS microchannels were fabricated to conduct simple proof of concept experiments for the design of a novel cartridge. To determine an ideal blood cleaning solution, bovine blood will be injected into the microchannels at a fixed flowrate and then cleaned using different reagents. The presence of blood and protein residue will then be assessed using trypan blue staining and fluorescently-tagged bovine serum albumin. In addition, a novel, reusable cartridge schematic was produced and modeled in AutoCAD and MATLAB. This design incorporated an additional inlet to allow for cleaning of the device and a novel streptavidin/biotin enzyme reservoir for reintroduction of fresh enzymes. Miniaturized analytical techniques as well as performance tests for each priority analytical method were incorporated in the final design. The proposed device shows promise for this NASA mission.

INTRODUCTION

The National Aeronautics and Space Administration (NASA) is currently developing the capabilities needed to send humans to Mars in 2030. The first trip to Mars is expected to last a total of three years. A space mission of this duration into deep space presents an array of unique problems, notably the monitoring of astronaut health. Experiments conducted on the International Space Station (ISS) have shown that the human body undergoes physiological changes in Space including a decrease in bone mass density, pooling of fluids in the lower extremities, and decrease in muscle mass. Furthermore, astronauts are confined to a small work environment with other personnel increasing the likelihood of illnesses to spread. Due to the above health risks, each astronaut's health must be monitored during the duration of a mission.

Blood panel tests are an important diagnostic tool used for the quantification of blood proteins and absorbed gases necessary for normal physiology. Quantification of these factors allow for screening of an astronaut's health. Current quantification methods utilize a hand-held cartridge system where a whole blood sample is loaded into the cartridge and probes within the handheld directly, or indirectly, measure the concentration of proteins and absorbed gases. Current cartridges used for blood panel testing are not reusable due to the deposition of residue from the blood, the sensitive nature of the enzymes used in the cartridge, and coagulation of blood within the cartridge. These one time use cartridges produce considerable biohazardous waste, take up space, and have a relatively short shelf life. The associated handheld devices used for quantification also have poor measurement sensitivity and reproducibility. Material and waste production must be minimized in the confined space of a spacecraft. Due to these limitations, a novel blood characterization system is required that is reusable, minimizes waste production, and has high measurement sensitivity and reproducibility.

Dr. DeVon Griffin was the assigned primary contact at NASA. The original design objectives given included designing a novel blood characterization system that would lower the required blood panel sample volume needed to run a test, increase the shelf-life of the new cartridge and the potential addition of more blood markers to test. Further along in the project, the needs of the project changed. The UA Enterprise, the senior design team group, was charged with the design of a novel, reusable blood panel cartridge system that either interfaces with the current standard technology at NASA or utilizes a new quantification technology.

After performing background research, the group focused on the development of a novel, reusable microfluidic device as detailed in the rest of this report. Due to the lack of lab space and laboratory resources, the senior design group could not test or fabricate the preliminary and final designs. However, the team's reusable cartridge design could be promising means of analyzing astronauts' blood components in space.

BACKGROUND INFORMATION

The i-STAT system is the leading technology currently used by NASA for quantification of blood factors. The i-STAT system requires a small volume of blood, varying from 11 μL to 95 μL depending on cartridge type, and allows for the quantification of over 25 different blood factors. The blood sample of interest is added to the well of the cartridge, the blood enters the main chamber of the cartridge via capillary action, and the blood interacts with a variety of enzymes to produce byproducts that are measured by ion- and impedance-sensitive probes contained within the i-STAT handheld (Abbot Point of Care 2014). The cartridges used by the i-STAT system are only single use due to use of capillary action to drive the sample, presence of only one inlet, and no means of clearing the adsorbed protein layer after completion of the analysis. There is also a large variation in i-STAT cartridges; there are seventeen different

cartridges, each of which can only measure a small subset of blood factors. This variation from cartridge type-to-type and within the same cartridge subtype results in significant handheld measurement variation.

Numerous limitations arise due to the design of the i-STAT system. i-STAT cartridges operate using unidirectional flow and therefore cannot be cleaned. Residual blood is left on the cartridge after each use. Sensitivity of the system is low as refrigeration time increases and the cartridges have a limited shelf life (Abbot Point of Care 2014). As stated above, the use of multiple different cartridges to measure a variety of different blood factors results in a great range of sensitivity and therefore measurement variation (Abbot Point of Care 2014). Lastly, there is a significant time delay for recognition of each new cartridge after loading it into the handheld, so blood samples must be loaded quickly to avoid coagulation (Abbot Point of Care 2014). Due to the above limitations and constraints of the current system, a new system must be adopted that uses a single cartridge that measures a range of high priority blood analytes in a highly accurate and reproducible manner.

Microfluidic platforms are a promising technology that can be utilized for NASA's replacement cartridge. A microfluidic platform provides a set of fluidic unit operations that are designed for a generic and consistent method for miniaturization, integration, and parallelization of biochemical processes (Nelson 2010). These systems allow for greater portability, higher sensitivity, shorter time-to-result, and less laboratory space consumption (Nelson 2010).

Two microfluidic platform types were investigated for the design of a novel cartridge. The first was a well-defined laminar flow system and the second was a droplet microfluidic system. Droplet microfluidic systems manipulate single droplets to allow for the compartmentalization, manipulation and measurement of individual samples quickly and reliably

(Giri 2011, Nelson 2010, Mark 2010). Therefore, hundreds of thousands of combinations or samples can be run in a high-throughput manner (~10^8 samples per day) (Giri 2011). These microdroplets containing small volumes (nanoliters) that allow for quick chemical reactions due to short diffusion distances and the use of chaotic mixing within the droplets during flow through the microfluidic system (Ling 2008). Thus, these samples are very uniform and allow for accurate quantification (Ling 2008). Despite the above advantages, many disadvantages were found. These systems utilize an oil-water immersion to generate single droplets that must be highly stable and uniform in volume and composition to yield accurate results. Depending upon the design of the cartridge system or the ratio of oil to water, the high degree of stabilization and uniform droplet size and composition is difficult to achieve (Ling 2008, Nelson 2010). Assays using these droplets must also be consistent in their washing, enzyme additions, quantification steps and final removal from the system. Furthermore, these droplet-based microfluidic systems are a new, emerging area of microfluidic research (Ling 2008). The current literature on microfluidic technologies is not as well documented as continuous, laminar flow systems. These limitations coupled with the fact that only a single astronaut will be using each cartridge made droplet microfluidic systems unnecessary. Therefore, the team opted for a laminar-flow system.

Well-defined laminar-flow microfluidic systems, on the other hand, are a promising approach since they are easy to implement, design, and scale-up in manufacturing. For biosensing applications, microfluids collected and then analyzed are derived from chemicals or biological samples from human tissue or bodily fluids (e.g. blood, saliva) (Ling 2008, Nelson 2010). Current miniaturized diagnostic tools used in the field are often integrated with these laminar-flow microfluidic systems, a major reason for adoption of this system (Ling 2008). Many difficulties may arise in the use of such a system. First, all of the preparative steps such as

collection of the sample, filtration of that sample, enrichment of the analytes, labeling, detection, and quantification must be done mostly before injection of the sample or the cartridge must implement many of these steps (Ling 2008, Nelson 2010). Also, laminar flow systems vary greatly along the path of flow making it so the entire flow field is dependent upon each section of the flow path. This inherent feature will introduce measurement deviations and inaccuracies (Ling 2008, Nelson 2010, Simmons 2006). Luckily, due to the large body of literature on laminar flow systems, design considerations for all major components such as valves, separators, micropumps, mixers and concentrators are elucidated (Ling 2008). Due to this major advantage and the fact that blood work of an individual astronaut does not require the advantages of droplet-based microfluidics, a laminar flow system was chosen.

For the duration of the project, the sample system studied was the original i-STAT system. The group studied this original system and the analysis methods used, and then designed a novel system that integrates these methods into a novel, laminar flow, reusable microfluidic system that also takes advantage of current miniaturized quantification systems.

The majority of the i-STAT tests are performed using enzymatic electrochemical assays. While it is necessary to remove the blood from the microchannels, it is unclear what effect a cleaning solution would have on the enzymes used to produce the measured analytes. After consulting with experts in biochemistry and electrochemistry, it was decided the best approach would be to rinse the microchannels with a mild surfactant followed by a rinse with water or PBS (Phosphate Buffered Saline). Since enzymes will likely rapidly degrade after wetting, literature on enzyme stabilization in a range of solvents was reviewed. After rinsing, one promising solution is to stabilize the enzymes in an ionic liquid. An ionic liquid's polarity, H-bond basicity, anion nucleophilicity, viscosity, and hydrophobicity can be tailored to the specific

enzyme being stabilized in the reusable cartridge system (Zhao 2010).

The primary analytes of interest for NASA are displayed in **Table 1** of the Appendix. The analytical methods that can be used to measure these analytes include ion selective electrodes, spectrophotometry, automated cell counting, and enzymatic electrochemical assays. Research was conducted by the team to determine how traditionally bulky analytical methods could be scaled down for a reusable cartridge system. The Moxi Z system pictured in **Figure 1** is a miniaturized cell counting technology that could easily be incorporated into a reusable cartridge. Moxi Z boasts 95% accuracy, and injection volume of 75 μL, and an 8 second analysis time (Dittami 2012). The cells from previous tests could be backflushed through a second inlet with a detergent to clean the pre-filter.

Spectrophotometry is a valuable tool for indirectly measuring enzymatic activity, concentration, and even metabolites of interest via the absorption of light at a specific wavelength. Current spectrophotometers, such as the Olympus 480 typically used by NASA Glenn Research Center, are desktop models. Even though these models have high reproducibility and a large range of measurable wavelengths (300 nm - 700 nm), the reliance on cuvettes and large sample volumes (3 mL or greater) to read sample absorbances makes this traditional method unfeasible for this application. Recent technology has scaled spectrophotometers to the miniaturized scale via the use of visible and ultraviolet light in conjunction with grating and photodiodes to measure absorbances of samples (Ling 2008, Nelson 2010). These technologies have great promise for this project.

PROJECT OBJECTIVES AND GOALS

With the above limitations and background research in mind, the project objectives and goals are as follows. The objective of this senior design project is to accurately monitor the

health of astronauts in space by designing a novel, microfluidic device that is reusable. This system will be used to measure analytes of interest found within the blood of the astronauts. Because a cartridge is soiled by the deposition of blood residue after each use, the primary goal of the project was to deduce how to best remove this residue with secondary goals in ensuring enzymatic activity over time, methods of introducing new enzymes, and a fully integrated design of the reusable, novel microfluidic platform. Due to the complex chemical analytical methods integrated into blood panel cartridges, each analytical component had to receive special consideration for cleansing.

To best remove the blood residue, The UA Enterprise designed an experiment with the goal of identifying the optimal cleaning solution for a blood panel cartridge containing simple PDMS microchannels shown in **Table 2** and **Figure 2** in the Appendix, respectively. A secondary goal of this experiment was to determine the best method of injecting the cleaning solution into the cartridge. Finally, the traditional blood panel cartridge design needed to be modified with an additional inlet and other considerations to enable effective backflushing for cleansing.

Due to the nature of the analytical methods used to measure some of NASA's primary analytes of interest, a cartridge that simultaneously measures all analytes of interest is currently not known. Therefore, another goal of this project was to incorporate new miniaturized technologies to substitute for traditionally bulky analytical methods.

METHODS/PROCEDURES/MANUFACTURING

After taking into consideration the current technologies used in laminar flow microfluidics and the need for a means of cleansing a reusable system after each use, The UA Enterprise came up with a preliminary design for a reusable blood panel cartridge. This design is

represented in an AutoCAD drawing shown in **Figure 3** of the Appendix. Annotations will be added for the final report. One key feature is the presence of a second inlet to backflow cleaning solution through. This feature is unique to the team's design and has not been found elsewhere in the team's review of the literature and patents.

In addition to designing a cartridge with the capability for reusability, the team sought to conduct an experiment to evaluate the optimal cleaning material to remove the blood residue. To accomplish this, a PDMS disc with several microchannels had to be fabricated, since PDMS is the most common material used for microchannel construction in cartridge systems. The complete PDMS making process is shown in **Figure 4**. The first step was getting the appropriate proportions of the PDMS solution to that of the PDMS curing agent, a 10:1 PDMS to curing agent ratio. Next, this solution was poured over a mold of micro channels, as shown in **Figure 5**, and then put into a degassing, vacuum chamber to remove trapped air in the liquid PDMS. This process took approximately 30 minutes. Leftover air bubbles were blown off the surface using a hand pump. The mold was then placed into a 65°C oven and the PDMS was allowed to cure for 6 hours.

When the PDMS had finished baking, a scalpel was used to remove the PDMS from the mold. The finished result was a PDMS disc containing indentations/channels corresponding to the mold. These discs were then cut into square blocks, holes were cut at the end of each lane, and the cut microfluidic device was plasma treated and then adhered to a glass slide. Plastic tubing can then be attached to the microfluidic chamber to create input and outputs for liquid. These final, crude microfluidic devices can be used for basic proof of concept experiments.

The UA Enterprise planned to conduct an experiment to identify the best cleaning solution to clean the reusable cartridge. Due to lab space issues, the cleaning experiment has not

yet been completed. The team has compiled a lab protocol, a list of materials with their corresponding MSDSs (Material and Safety Data Sheets), and a chemical hygiene plan approved by The University of Akron's Health and Safety Department. The experiment involves injecting bovine blood at a fixed flow rate using a syringe pump into a PDMS microchannel. The cleaning material of interest will then be run through the channel at a fixed flow rate. The PDMS disc will then be plasma treated and adhered to a glass cover slide. The microchannel will be observed under a light microscope using trypan blue as a staining agent to qualitatively assess the presence of blood residue. The team aims to complete the cleaning experiment as soon as lab space is available.

PERFORMANCE TESTING

Enzymes are typically stored in a lyophilized, solid powder form. Once wetted by a fluid such as water, the enzymes may undergo rapid degradation. When the enzymes in an electrochemical enzymatic assay begin to degrade, the amount of product molecule produced will be reduced, and the detected level of the compound of interest in the blood will be artificially low. One way to ensure that the new blood panel cartridge is delivering accurate results after repeated use is to inject a standard compound after each use. For example, injecting Aspartate Aminotransferase (AST) at a concentration of 37 U/L the first time may evoke a 100% response and yield the correct measurement of 37 U/L. However, the second use may only evoke a 50% response, and therefore the instrument would incorrectly display 18.5 U/L as the result. The way in which the response weakens, however, is quantifiable. In the example shown in **Figure 5**, the measured response to a repeated 37 U/L standard AST injection decayed exponentially. When measuring a sample of blood with an unknown concentration after a known number of uses, the correct concentration could be interpolated and the actual value could be

displayed. This procedure could be used to validate all the enzymatic measurements, including glucose, AST, and Alanine Aminotransferase (ALT).

The number of cycles or cartridge uses that leads to decreased enzymatic activity must be determined for each enzyme. The enzyme activity can be quantified by measuring the amount of substrate processed by the enzyme per minute as measured using UV-Vis spectrophotometry at the wavelength of maximal absorption of product and a product standard curve. This will allow for the quantification of the amount of product released by the enzyme within a given time period. This value will be the measure of the enzyme activity.

The enzyme activity for each enzyme should be measured for each cycle until a noticeable decrease in enzymatic activity is seen. An example graph of such a decrease in enzyme activity can be seen in **Figure 6** in the Appendix. The number of cycles that leads to a diminished enzymatic activity will mark the maximal number of times the cartridge can be used before the enzymes must be replaced or a whole new cartridge is used by the astronaut.

Measurements involving automated cell counting include white blood cell count, hemoglobin, neutrophils, and lymphocytes. A calibration kit with known cell quantities could be used to ensure that the instrument's measurements are accurate. The main concern with a reusable system is that it is necessary to completely wash away residual cells so that the measurements after the first measurement use are not inflated. If completely washing away the cells from a prior use is not possible, the cartridge can be "blanked". For example, if there were 12 cells remaining in the counting area from the first measurement, the instrument would ignore those 12 cells and start counting the new cells starting from zero.

Compounds measured with an ion selective electrode include potassium and sodium. The electrodes can be cleansed with a pH neutral solution such as water. To ensure that the electrode

is clean and not corroded, standards of a known ion concentration could be run. If the electrodes are in working order, the measured values will closely match the standard concentration.

Creatinine is one analyte in the blood that can be measured spectrophotometrically. The oxidation of p-methylamino phenol sulfate (Metol) in the presence of copper sulfate and creatinine yields an intense violet colored species with maximum absorbance at 530 nm. This method of constructing a linear calibration curve by injecting a dilution series of a creatinine standard has been validated (Krishnegowda 2013). The same-day precision of this test normally ranges from 2.5-4.8% (Krishnegowda 2013). The probable reaction mechanism forming the imine group that causes the purple color and the calibration curve obtained from Krishnegowda 2013 can be seen in **Figure 7** and **Figure 8** in the Appendix. To maintain the same accuracy in a reusable system, the system would have to be thoroughly cleansed to make sure no leftover coloration carries over to the next sample. This could be validated by remeasuring the same sample after cleaning. The two measurements should be within a reasonable margin of error.

FUTURE DIRECTIONS

In order to ensure reusability of this device, a means of selectively introducing the same concentration of enzyme after a full cleaning must be developed and tested. We recommend the use of a highly selective biotin and streptavidin system for the introduction of new enzyme on a glass surface.

The interaction of biotin and streptavidin has been exploited for use in many protein and nucleic acid detection and purification methods. Biotin labels are small, stable and rarely interfere with the functionality of the labeled molecule. Biotin has a very high affinity to streptavidin molecules. This strong non-covalent interaction can be exploited in a manner so as to introduce the same number of enzymes on a given surface. In a microfluidic blood panel

system, this may allow for reusability of the same chip system and thus lower the amount of waste.

A glass surface can be conjugated with immobilized streptavidin at a specified density. Biotinylated enzymes are then introduced under physiological flow and the enzymes are anchored to the glass surface via the biotin-streptavidin interaction. If new enzyme needs to be introduced after enzyme activity has fallen below acceptable levels that will affect the blood panel's final analysis, the glass surface can be washed with a basic solution (pH 10) and the biotin-streptavidin interaction will dissociate and the old enzyme will be washed off of the surface. After washing the surface with phosphate buffered saline (PBS) or deionized water so as the immobilized streptavidin is at physiological pH, the process can be repeated to add additional enzymes. This process of washing and introducing new enzymes on a glass surface containing immobilized streptavidin is shown in **Figure 9**.

This system has many advantages over what is currently used on the market. The binding of the streptavidin to the biotinylated enzyme is highly specific and thus will yield a constant enzyme loading onto the surface. The major disadvantaged for such a system is cost; each enzyme of interest will have to biotinylated and the resulting complex enzymatic activity will have to be investigated prior to its use in the microfluidic system.

With the above design considerations in mind, the senior design group is currently in the process of making a 3-D model of the final microfluidic chip design in both Solidworks and MATLAB. In Solidworks, a 3-D representation of how of the parts will fit together will be shown in multiple views (aerial, left/right, from below, and stacked). MATLAB modeling capabilities and Solidworks will be used to model the fluid and pressure forces as well as the mechanical properties of the entirety of the chip. If time allows, designs for the miniaturized

coulter counting system and spectrophotometer will be included in the final Solidworks design.

FUNCTIONAL REQUIREMENTS

The functional requirements taken into consideration during the duration of this research and design project are listed in **Table 3** of the Appendix. These requirements must be met in order for the proposed device to be utilized in space.

CONSTRAINTS AND LIMITATIONS

One major limitation of the project was that all design work for a device to be used in zero gravity conditions had to be done on Earth. The system implemented had to take up minimal space, add minimal additional weight, and produce minimal waste. Because the planned Mars mission is expected to take 3 years, the device must have a shelf life of at least 3 years. The background research and design work had to be completed within a two semester timeframe with a $500 budget. Finally, The materials used for the reusable cartridge must be nontoxic and non-hazardous. The accuracy of the system must also be maintained after multiple uses.

TIMELINE

A Gantt chart was utilized to keep track of all phases of the project. During the first semester, background research on the current i-STAT system was conducted and proof of concept experiments for the microfluidic device were brainstormed and evaluated. In the second semester of this design project, a blood and protein adhesion proof of concept experiment was devised. Due to lack of laboratory resources, this experiment and others could not be conducted. From research conducted in the first semester, a design of the novel, microfluidic device was

drafted. Modeling of this design is currently being done in MATLAB, Solidworks and AutoCAD, and will be included in the final report.

BUDGET

During the course of this design project, the major experiment of interest was to determine the ideal cleaning agent or detergent that could be used for a reusable cartridge. The items listed in **Table 4** were procured for the proposed cleaning experiment whose result would elucidate the best cleaning agent for the system.

Other supplies and lab space was generously supplied by Dr. Hossein Tavana from the Biomedical Engineering Department at The University of Akron. Even though the experiments were cancelled due to the inability to find lab space, the cost to conduct the experiments was $106.74. This is excluding the cost of any machinery that would be required in order to make molds and treat the PDMS.

In order to independently manufacture these PDMS microfluidic chambers, a 65^{o}C oven, oxygen plasma treatment machine, vacuum pump, cutting apparatus, and an surface contact measurement device would be required. The oven used for curing the PDMS elastomer kit would range from $500 to $800. The oxygen plasma treatment device used to both make the plastic hydrophilic and sterilize the plastic device would cost $6000 to $10,000 depending on the model. Lastly, the surface contact measurement device could be made using <$500 worth of equipment. The surface angle measurement device is of upmost importance; the measurement of the contact angle made by a droplet of water on the PDMS surface of the microfluidic device is a direct measure of the hydrophobicity of the surface. This hydrophobicity measurement can be used as a means of quality control during manufacturing of numerous devices.

The rest of the costs associated with this device, besides those listed above, are related to exploratory research in the use of enzymes for this system. For testing reusability of enzymes, a biotinylation kit can be acquired from Life Technologies, Inc. to biotinylate the enzymes of interest. Enzymes that will be tested and/or used for this system include Serum Glutamate Oxaloacetic Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT). Streptavidin will also be procured from Life Technologies, Inc.

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Appendix

Table 1

Analyte	Full Name	Analytical Method	Analytical Range[1]
Blood			
K^+	Potassium	Ion-selective electrode Olympus 480 indirect method	3.8-5.2 mmol/l
Na^+	Sodium	Ion-selective electrode Olympus 480 indirect method	135-144 mmol/l
Creatinine		Spectrophotometry Olympus 480	0.6-1.3 mg/dl
Glucose		Enzymatic Olympus 480	71-99 mg/dl
WBC Count	White Blood Cells	Automated cell count Coulter LH750	3.7-10 M 3.4-10.6 F
HgB	Hemoglobin	Automated cell count Coulter LH750	12.6-17 g/dl-M 11-15.3 g/dl-F
Neutrophils		Automated cell count Coulter LH750	43-72%
Lymphocytes		Automated cell count Coulter LH750	17-43%
AST (SGOT)	Aspartate Aminotransferase (Serum Glutamate Oxalo-acetic Transaminase)	Enzymatic Olympus 480	7-37 U/L
ALT (SGPT)	Alanine Aminotransferase (Serum Glutamate Pyruvate Transaminase)	Enzymatic Olympus 480	2-42 U/L
Urine			
Urine Leukocytes		Reagent strip reflectance Clinitek Advantus	Negative
Urine Proteins		Reagent strip reflectance	Negative
Urine Blood		Reagent strip reflectance	Negative

Table 1: Major analytes that need to be measured by this device and the analytical method used to determine their relative concentrations in the blood.

Figure 1

Figure 1: Image of the Coulter Counter Moxi Z miniaturized cell counting system. This commercially available system demonstrates the potential in miniaturized devices that can be directly translated to the team's microfluidic system.

Figure 2

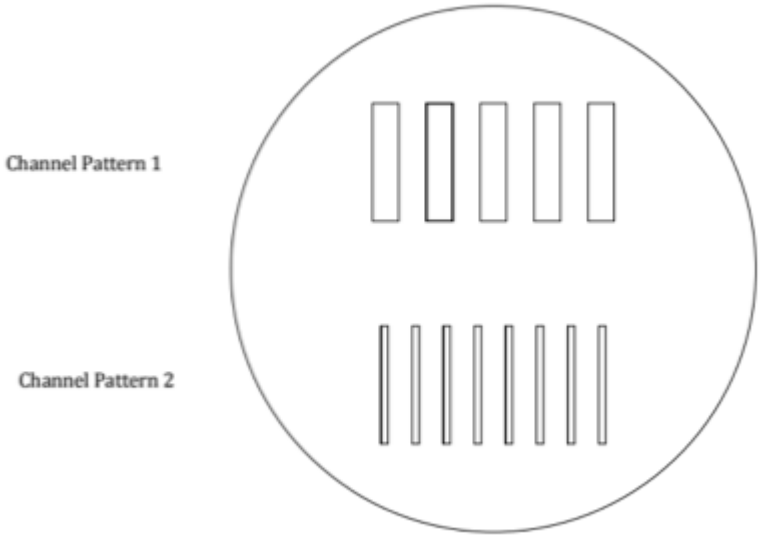

Figure 2: Details the layout for the basic microfluidic system used for preliminary tests. Groove Pattern 1 has a groove width of 3800 µm and a gap width of 4000 µm. Groove Pattern 2 has a groove width of 1000 µm and a gap width of 3500 µm. Two different groove patterns were used to demonstrate the effects of differing channel sizes.

Table 2

Fluid Through PDMS Microchannel	Residual Blood Particles
Blood	
Blood and then Deionized Water	
Blood and then Hydrogen Peroxide	
Blood and then Acetic Acid	
Blood and then Iodophors	
Blood and then Saline	
Blood and then Peracetic Acid	

Table 2: For the proposed experiment to determine the optimal cleaning solution for the device, the above table would have been used to record results. The residual blood particles would have been determined via light and fluorescent microscopy. In fluorescent microscopy, BSA, bovine serum albumin, with fluorescent tags would be flowed through the device. The following fluids would then be used to try to clean the device. Residual fluorescence would be noted as compared to a control group. - The UA Enterprise

Figure 3

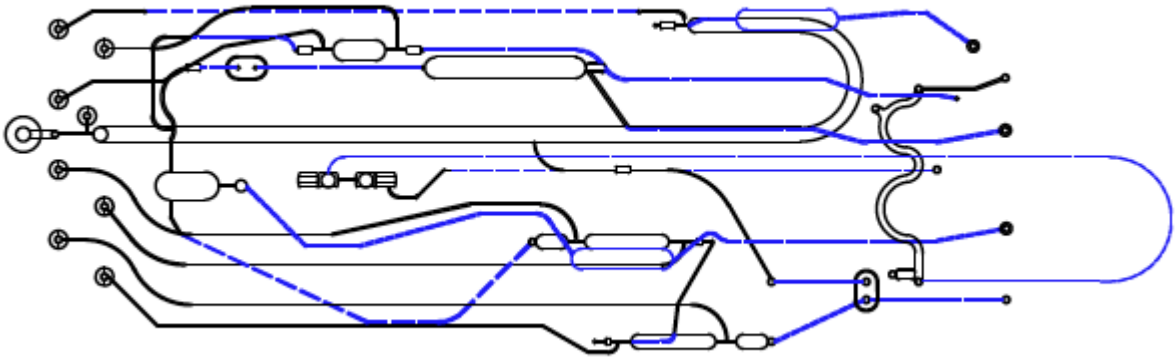

Figure 3: AutoCAD Drawing of Reusable Cartridge *In Progress*. - The UA Enterprise

Figure 4

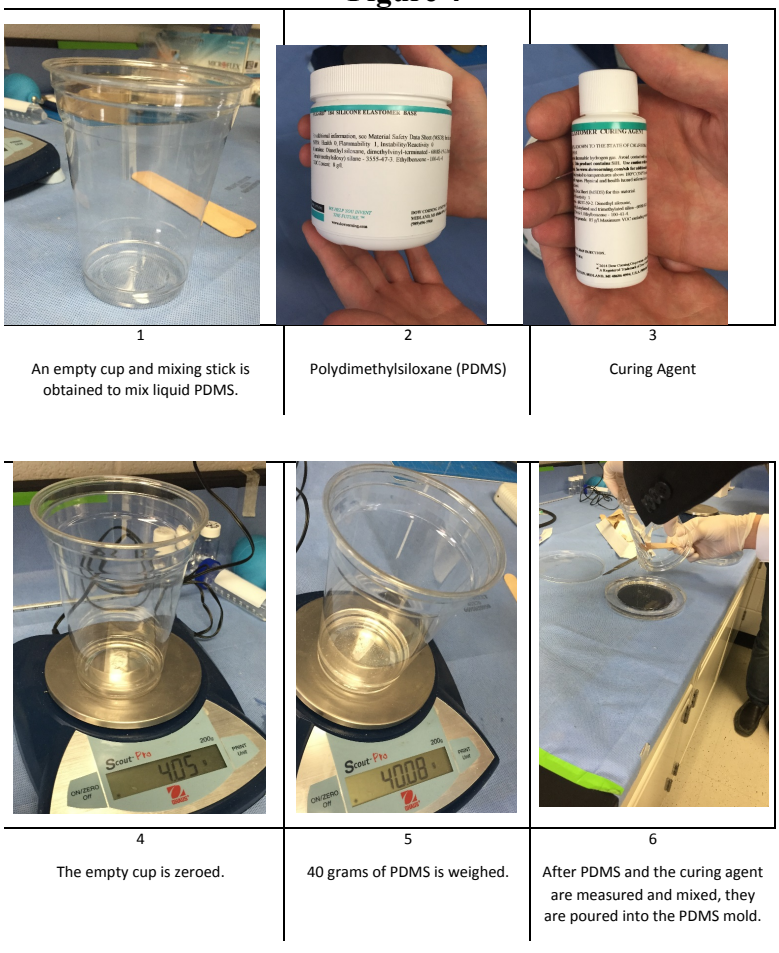

Figure 4: First six steps of the PDMS molding procedure, from the removal of air from the liquid PDMS to the fixation of PDMS via using the oven. - The UA Enterprise

Figure 5

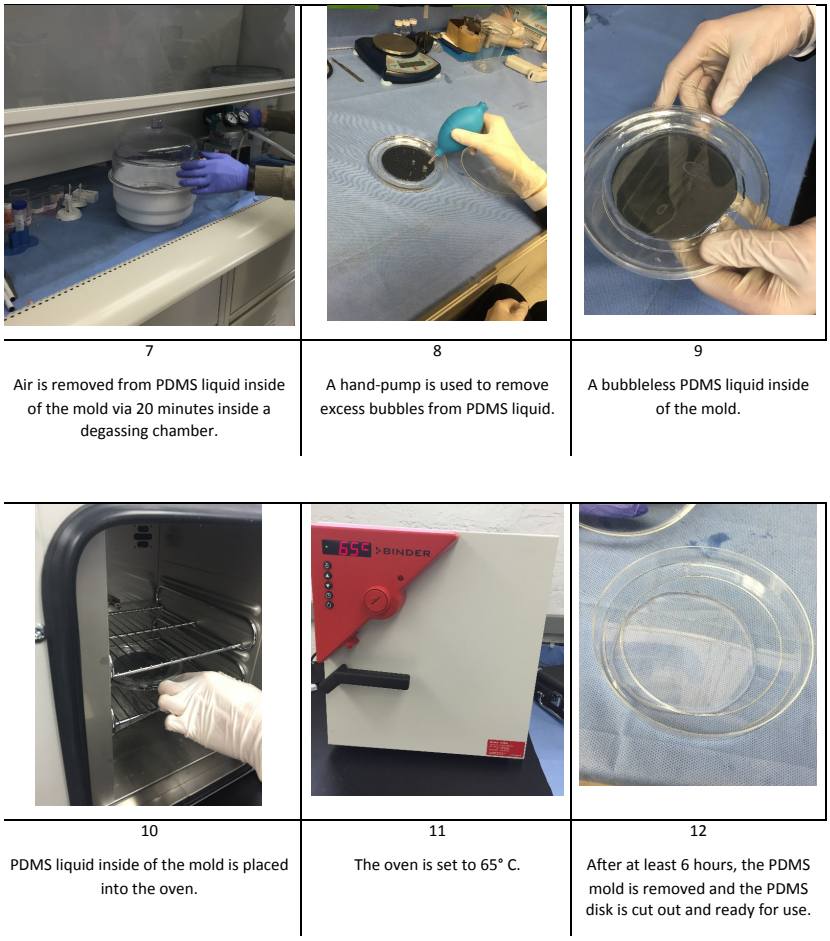

Figure 5: Last six steps of the PDMS molding procedure, from the removal of air from the liquid PDMS to the fixation of PDMS via using the oven. - The UA Enterprise

Figure 6

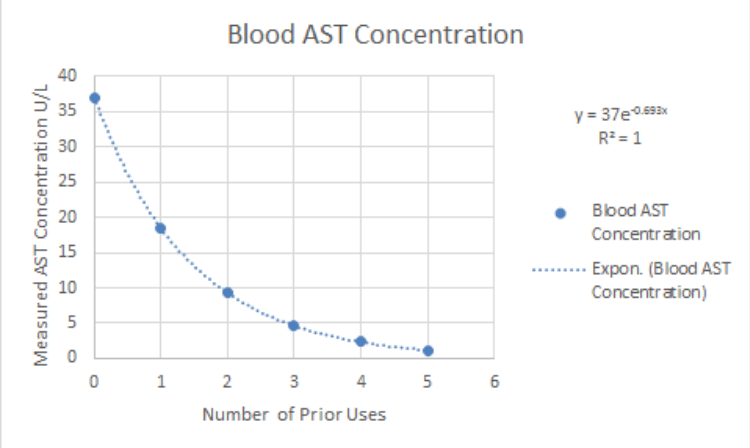

Figure 6: Example of diminishing enzymatic activity after multiple device uses as shown by lowered ability to determine the concentration of Aspartate Aminotransferase. - The UA Enterprise

Figure 7

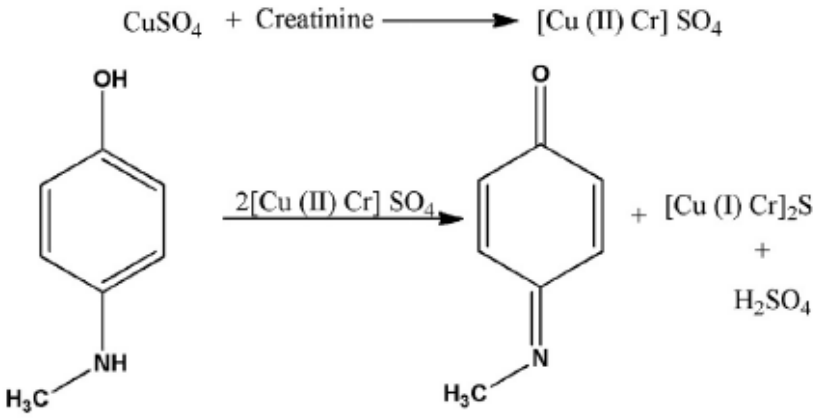

Figure 7: Mechanism for the formation of a violet colored species from Creatinine and Copper Sulfate with a maximal absorbance at 530 nm. (Krishnegowda 2013)

Figure 8

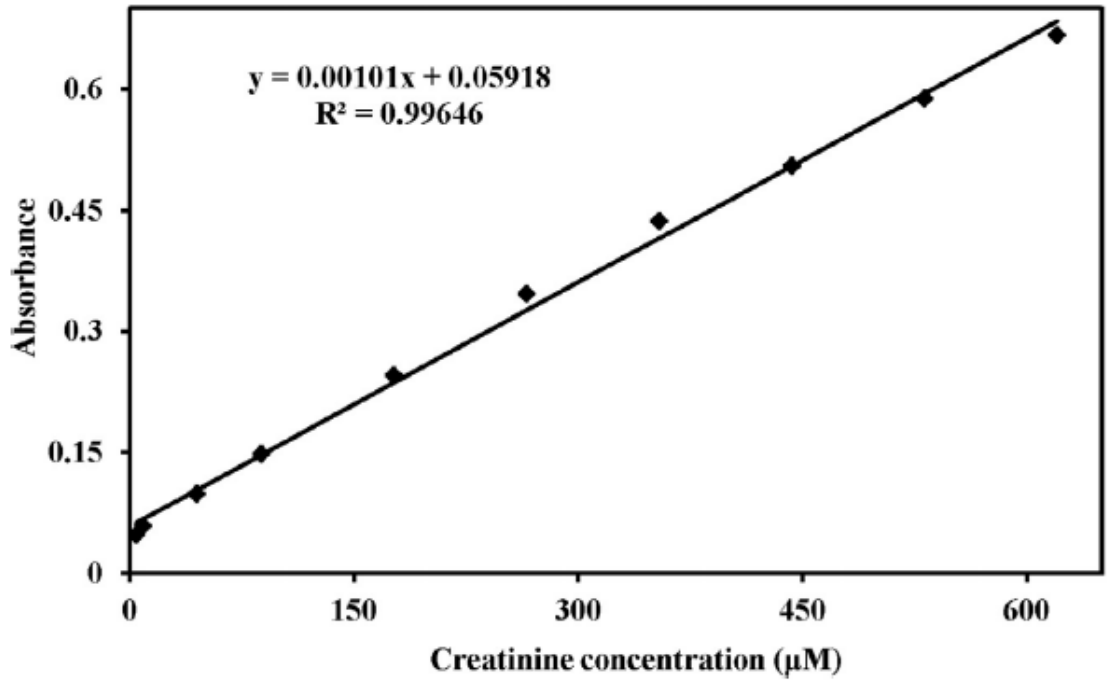

Figure 8: Graph of absorbance versus creatinine concentration as measured by spectroscopy. (Krishnegowda 2013)

Figure 9

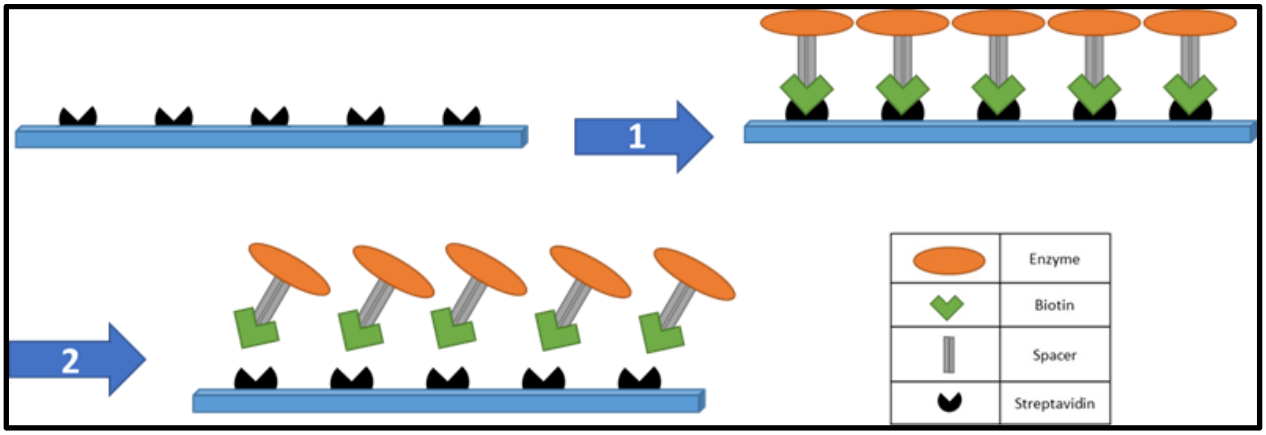

Figure 9: Immobilized streptavidin is washed with a solution containing biotinylated enzyme (Step 1), resulting in a relatively consistent density of enzyme on the surface. If the old enzyme loses its enzymatic activity, the surface can be washed with a basic solution to dissociate the strong streptavidin-biotin interaction.

Table 3

Functional Requirements	Relative Importance
Reduced system size	5
Detergent used clears bio-film	9
System allows for reintroduction of enzymes	10
System produces non-hazardous waste	9
System is sterilizable	9
System shelf life (3+ years)	9
System cycle life (100+ cycles)	9
System does not leak; fluids go through inlet and outlet	9
System utilizes pump system to drive liquid	6
Polymer utilized in system is highly stable in liquid	8
Systems allows for measurements that are highly reproducible and accurate	10

Scale: 1-10
1 = Low priority
10 = High Priority

Table 3: The above functional requirements were first supplied by Dr. DeVon Griffin from NASA Glenn Research Center. During the duration of the project, each requirement was taken into consideration in a manner proportionate to its relative importance to the entirety of the project.

Table 4

Item	Cost ($)
Hydrogen Peroxide	1.91
Sylgard 184 Elastomer Kit	62.90
Chlorox Bleach, 30 oz.	6.99
BTF Iodophor Sanitizer, 16 oz.	14.45
Acetic Acid, food grade, 950 mL	20.49

Table 4: Reagents used in the proposed design experiment to determine the optimal cleaning solution for the reusable cartridge.