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Microfluidic E. coli Detection

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ENTITLED

MICROFLUIDIC *E. COLI* DETECTION

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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Microfluidic *E. coli* Detection

By

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Senior Design Project Report

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Abstract

In both developed and developing countries, there is a need for a fast diagnostic system to detect pathogens within a fluid sample. In developing a microfluidic platform, which utilizes a microfluidic chip and an optical detection method, doors may be opened for new methods of determining pathogen concentration in fluid. Most biological reactions are not instantaneous. A flow-controlling mechanism with no power requirement may be implemented in the microfluidic platform. As a proof-of-concept, our device uses a microfluidic chip, smartphone, and microlens to detect *E. coli* concentrations in water. The detection method is based on the latex agglutination assay which relies on visual observations and judgment to determine the presence of pathogens in the water sample. Our approach provides a quantification of the traditional latex agglutination output, and the lower detection limit (10^5cells/mL) is competitive with that of the traditional agglutination method. In developing such a platform, a cheap and effective detection test for people in developing countries can be available worldwide for easy determination of whether or not a fluid sample is safe for use, and with several modifications, this platform could potentially be used to detect different pathogens, simultaneously.

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Chapter 1: Introduction

1.1 Background

Waterborne disease caused by interacting with contaminated water remains an important global health issue, particularly in developing nations. Common waterborne contaminants include *Salmonella*, *Vibrio cholera*, and *Shigella dysenteriae*. Another significant source of contamination is *E. coli* O157:H7 bacteria. Of the many different strains of *E. coli*, this specific pathogenic strain causes hemorrhagic diarrhea, and in extreme cases will lead to kidney failure. Testing water samples for *E. coli* O157:H7 contamination is conventionally performed in laboratories off-site with expensive equipment and results take days or weeks to return. This makes the technologies used not applicable for testing water supplies in rural regions. However, in recent years, there has been a push towards point-of-care testing (POCT)—which allows for a quick, on-site diagnosis in a variety of biomedical areas—particularly within developing nations. In seeing this push, the World Health Organization (WHO) has established seven guidelines for the development of diagnostics in resource-limited settings. They say the diagnostic tests for the developing countries should be "ASSURED" – affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to those in need [1]. With these guidelines, we proposed to develop a low-cost, semi-autonomous, miniaturized fluidic device capable of detecting *E. coli* in fluid samples, which will be useful for environmental monitoring, especially in developing nations. This device would be usable by personnel with minimal training and minimal equipment at the testing site. Our device utilizes a microfluidic chip capable of driving fluid flow with capillary force, eliminating the need for an external pumping system. Similarly, the detection method used with the microfluidic chip is designed to require no additional equipment. This will ultimately limit the overall cost and complexity of the system and allow the device to comply with the "ASSURED" guidelines of the WHO. A sample of the microfluidic chip used to drive the fluid by capillary flow is given in Figure 1.1.

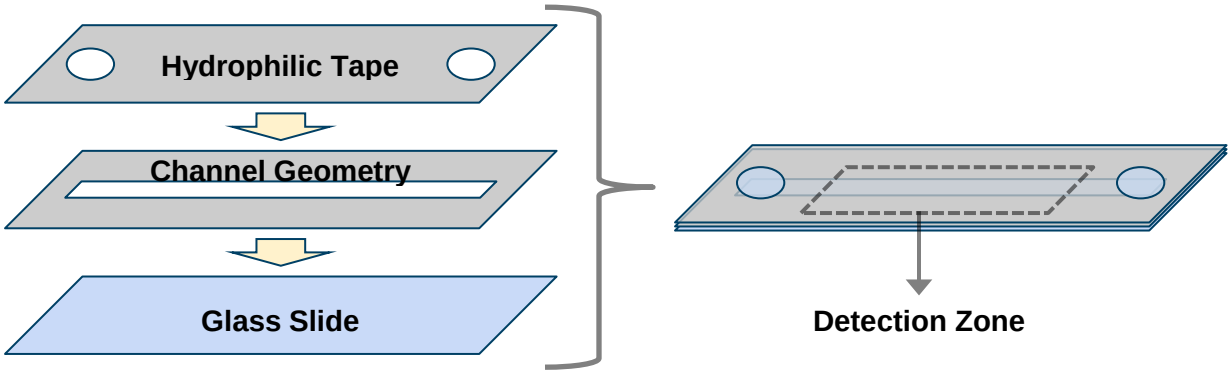

Figure 1.1: The basic concept of the capillary-driven fluidic chip. The middle layer of hydrophilic tape may be adjusted to fit the needs of the project in creating a fluid control system.

The system relies on an agglutination method that uses latex particles, which are coated with antibodies that specifically bind to antigens found on *E. coli* O157:H7 cells. Agglutination, or the process of clumping particles by binding antibodies to antigens, magnifies the signal produced by the pathogenic cells, as the agglutinates are much larger and easier to see than the cells themselves. By using a microfluidic system to gather these agglutinates in a closed environment for analysis, it is possible to quantify the concentration of *E. coli* cells within a fluid sample. Figure 1.2 shows a diagram of the process of agglutination and depicts formed agglutinates.

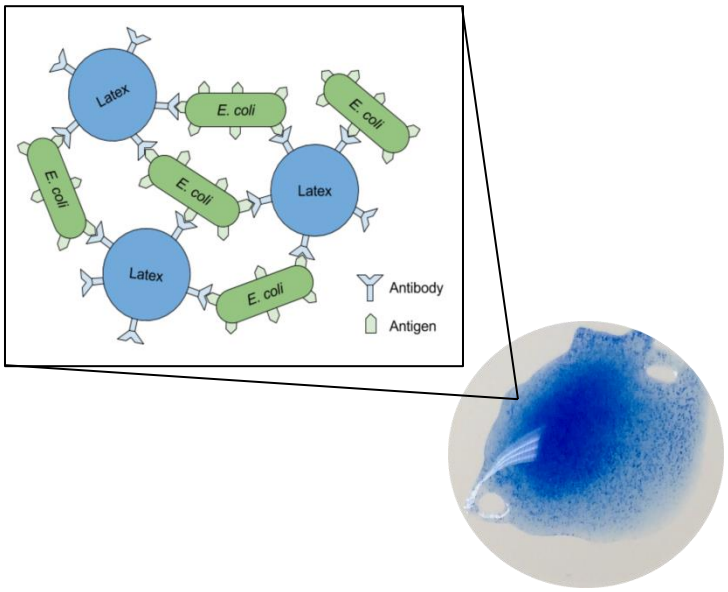

Figure 1.2: Process of agglutination and an image of formed *E. coli* O157:H7 agglutinates.

Additionally, our device can be refitted to assist in diagnosing the cause of various illnesses by functionalizing latex particles to specific pathogens. The WHO notes syphilis and hepatitis A and B to be major problems in Africa [2]. These diseases could be tackled using our device with slight modification. If a patient walks into a hospital with severe gastroenteritis, the cause could be one of many. A simple test with this device could potentially lead to quick and specific detection of the pathogen at fault. This will reduce the cost of medical care and the pending time for results.

The fluidic platform developed in this research provides a low-cost, user-friendly tool to detect *E. coli* in fluid samples, which can directly benefit rural regions where there are no programs for testing water supplies. Allowing such regions to test their water before using it will enable the less fortunate to avoid illness from the use of contaminated water, improving the health of these communities. This ability—to sustain good health—is a benefit to humanity in general, as staying health is not only for the fortunate, but for the people in developing countries as well. If our device were to be commercialized and reach more people, the overall safety of the public could improve.

1.2 Literature Review

In the developed world, detecting pathogens in water and diagnosing disease is often performed with expensive high-tech equipment and methods. This is not practical in the developing world, where the high cost of such detection methods often prevents their usage. In response to the limitations of cost, as well as constraints involving access to electricity and trained personnel, cheap and robust microfluidic diagnostic chips have been developed. Numerous methods of driving the fluid flow within the microfluidic chips have been utilized, including electrically powered pumps, gravity, and capillary flow [3]. Of these methods, capillary flow is the cheapest and easiest to use because it does not need additional electronics and can be built into the design of the channels themselves, requiring minimal user interaction to prepare the flow [4].

Research has also shown that fluids can be held in microfluidic chips without any external assistance [5]. In our product, there is a need for a fluid control valve, as the pathogens in the fluid need time to bind with other particles present in the fluid to amplify the signal for the detection method to function properly. It is desired to have as little human interaction as possible in handling the valve during a test, as it is important to have a consistent incubation time among tests to provide consistent results. Though there are research projects that have shown evidence of automatic control valves [5], it has been noted that these valves work on the nanoscale, whereas our product will be functioning on the microscale. In seeing this, attempts were made towards an automatic valve; however, a consistently working valve with limited user interaction is the ultimate desire for our product.

In reviewing research and literature regarding valve design, numerous concepts have been analyzed and ideas for how our design should look and function have been gained [6]. One of the reviewed microvalve documents provided a framework on how derivations for a microfluidic valve should be completed for a consistently functioning automatic stop valve. Though the reviewed valves seemed promising, the proposed designs were not confirmed to function 100% of the time. Therefore, designing a valve that functions properly in every test would be a large improvement to the reliability of microfluidic chips.

Possible detection methods were also assessed in reviewing microfluidic literature. A light-sheet microscope was found to be particularly useful for our project, as it provides us with one method to optically detect the pathogens in our chip [7]. It also provided the idea of using latex agglutination in order to amplify the signal of the cells, making them easier to detect for any detection method in general. With our preliminary review of literature in the field, we felt that it was worthwhile and possible to pursue the idea of creating a microfluidic platform capable of detecting *E. coli* in fluid samples.

1.3 Project Objective

The objective of this design project is to develop an *E. coli* detection method with a microfluidic platform capable of analyzing a fluid sample for the concentration of the *E. coli*. In order to amplify the *E. coli's* signal for the detection method, latex particles will be used to bind to the *E. coli* and create agglutinates. The following three detection methods were considered for our design:

- Optical Detection
- Electrical Impedance Detection
- Chamber Analysis Detection

Also, the microfluidic chip is desired to have the following qualities:

- Low cost
- Accurate
- Easily Distributable
- User-Friendly
- Fast Detection

Chapter 2: System Level

2.1 Customer Needs

While designing a consumer product, it is important to take into account the thoughts and needs of the customers. To do this, surveys and questionnaires may be used strategically to gain insight on how to make the design of a product more appropriate for the users. It is also important to define the primary and secondary users of the product, in order to create a hierarchy of the necessary design aspects.

For our project, a questionnaire was created to gain the insight necessary to better the product. The primary users were identified as people and organizations in developing countries, while meat handlers and water distributors were deemed the secondary users of the product. Because of the diversity among the users, the questions in the questionnaire were designed to be able to be answered by each of the users.

Unfortunately, after consulting a variety of resources, no contacts to a developing country could be found. Also, because food handling is such a confidential industry, as there are many requirements and standards a company needs to pass in order to sell food, all companies reached out to were unwilling to share information about the tests they performed to pass the required standards for their produce. Despite this lack of data, articles about the necessity for diagnostic technology in developing countries were found and used to answer some of the questions our team had about the customer needs [1, 2]. The answers to the following questions may be found in Table F.1.

1. Is there a need for a portable, contamination detector for water in developing countries?
2. How would our product be used? In what situations?
3. Have there been previous detection devices made? What were some of the problems?
4. What are the biggest challenges for providing healthcare products to developing countries?
5. What are the most desirable qualities of a contamination detection device?

6. What could be improved upon our proposed design or previous detection methods?
7. Which is more important: a quick detection time, a low cost, or a small size?

After analyzing the answers to these questions, a few conclusions were made on the importance of the different aspects of the senior design project. To begin, it seemed as though affordability was the most important quality of the project. This was because if the device were cheap, it could be distributed more easily and more people would benefit from it. Rather than making an expensive, highly technological detection device, it seemed as though people in developing countries would rather have a detection device than no device at all. This was one of the current problems for providing detection methods to people in developing countries: the available devices were too expensive and the supplies needed for the detection devices were highly technological. Therefore, when designing our detection system for *E. coli*, it was important to take into account the final product's cost. Because of this, the microfluidic chip was given a design specification of costing less than one dollar and the product as a whole was to cost as little as possible, in order to make the product affordable for people in developing countries.

The next most desired quality in the detection device was simplicity. Many of the devices already available are not only too expensive, but too complicated and required extensive knowledge and training to use. Since people in developing countries lack the necessary knowledge and training to use such technologies, the detection methods available are simply not used.

One of the last necessary aspects of the design project was a quick detection time. As stated in the answers to the questions, long detection times gave rise to other problems, such as results becoming mixed-up in health clinics in the developing countries. Also, if there were a moment where water was needed immediately, time may not be available and a long wait time would make the detection device ineffective to the urgency. In seeing this, the detection time target specification for the design project was less than ten minutes in order to provide a quick and reasonable detection time.

Other than these important aspects, the device was designed to be robust and reliable. In making the product robust, it was desired that the product be able to withstand the harsh environments of developing countries. To accomplish this, the materials of the product should not perish quickly or break easily. The product should also be able to be used almost anywhere, as one of the key features of our product is that a laboratory is not necessary for utilization. In making the product reliable, results from the device should be accurate enough that users can trust them. To implement this level of reliability and trust in the product, the microfluidic chip in the final product was given a design specification to work 9 times for every 10 chips. Ultimately, in gathering information about the *E. coli* detection system, a great amount of insight for the advancement of the senior design project was gained and used to better the design for the customers.

2.2 Design Sketch

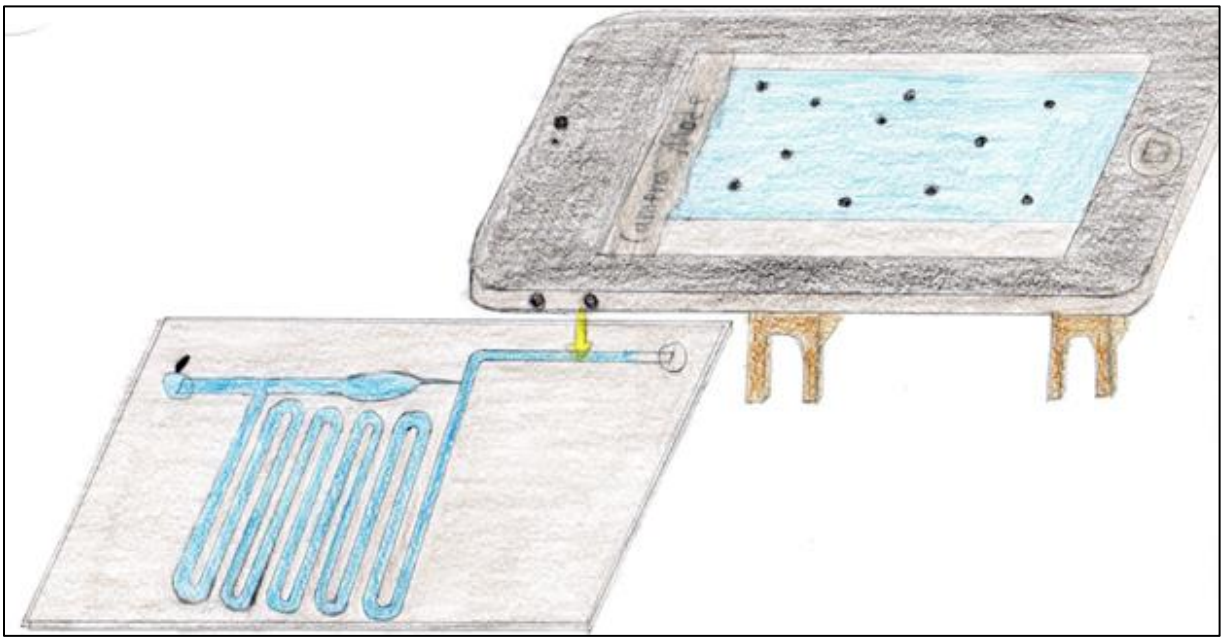

Figure 2.1: A simple sketch of the predicted design, including the microfluidic chip and the optical detection method using a smartphone. (Drawn by Kyle Pietrzyk)

Figure 2.1 depicts an optical detection system design that was specifically created with the needs of developing countries in mind. A smartphone was used in this detection method because

smartphones are one of the fastest growing technologies in the developing world. Designing the produce in this way allows people in developing countries to actually use the microfluidic chip and not be barred out by expensive costs or extra technologies.

2.3 Functional Analysis

The device consists of a microfluidic chip and an optical detection algorithm that can be compiled into a smartphone application. A small water sample is mixed with latex particles, which are designed to agglutinate with *E. coli*, inside the microfluidic chip. The agglutinates then flow through a channel where a smartphone with a magnification attachment records a video of the flowing fluid. The recorded video is processed by our algorithm, which calculates the change in light intensity of the video before and after the fluid sample flows through. The change in intensity is then used to find the water sample's concentration of *E. coli* using a correlation curve.

When analyzing the functionality of the device, it was important to have a decent understanding of each part of the system. One of the most important parts of the device was the microfluidic chip. The chip was made of three layers of different materials. The bottom layer was made of glass, which provided a sturdy and flat foundation for the fluid as it flowed through the microchannels in the second layer. The second layer was made of a plastic tape, which had microchannels carved into it to guide the water through the chip. The top layer was made of another tape with hydrophilic adhesive, which could pull the water through the channels of the microfluidic chip. Two holes were cut into this layer at the inlet and outlet of the microchannels. Since the middle layer was very thin and had a small contact area with the fluid flowing through the channels, the material of the top layer was very important, and it was altered until the fluid was pulled through the microchannels at a desired rate. Therefore, one of the biggest decisions made in creating the microfluidic chip was the material of the top layer.

Another function the microfluidic chip performed was containing the sample fluid in a mixing chamber where a binding agent—latex particles with antibodies for *E. coli*—was given time to bind with the pathogen in the sample fluid. The fluid was able to be held in the mixing chamber

with a microvalve, which was capable of stopping the flow through the chamber using the surface tension of the fluid. The end of the microvalve could then be connected to the side of another microchannel, causing fluid flowing through the alternative microchannel to release the fluid in the chamber when present and continue through the chip. In using a microvalve to stop the flow, the product could be simplified, as no extra components would be necessary to stop the flow of the fluid for mixing.

The last critical functioning part of the project was the detection method used to analyze the pathogen concentration in the flow. There were many ways this task could have been completed, as described in Appendix D, but one of the most promising ways was through optical analysis. In this method, the flow was to be analyzed with a smartphone and a microlens attachment by looking for agglutinates in the fluid sample. In doing so, a smartphone application could be created to analyze a video of the flow in the channel and calculate the concentration of the pathogen in the fluid. With these functioning parts of the product, the product was predicted to work with slight modifications based on experimental results.

In thinking about the functioning parts of the product, it was important to list the inputs and outputs of the product in order to gain an expectation for what was necessary to use the product and what should be expected from the product. Since the product was designed to be as simple as possible, the only necessary input for the product to work was the sample fluid mixed with latex particles, granted that the detection method was set up and in place. If the sample fluid were put in the correct spot on the microfluidic chip and the detection method was ready to calculate the concentration, then the inputs for the system were already obtained. As for the outputs of the system, one should expect the concentration of the sample fluid, which would be calculated with a smartphone. These were the inputs and outputs of the final product.

2.4 Benchmarking Results

Although the proposed product is rather unique in some aspects, there are already other available consumer and research products that accomplish similar goals. However, these products do not have all the desired characteristics we believe are necessary for a water contamination detector in

a developing country. Characteristics such as a low detection time, low cost, lack of special equipment, and portability should all be present in a pathogen detection system for a developing country. As discussed in the following products, some of these characteristics are greatly enhanced while others are forgotten.

In the first research product, a complementary metal-oxide-semiconductor (CMOS) imager was used with a microfluidic chip to detect small particles flowing with the fluid through the chip's channels [7]. After sample fluid was placed at the inlet of the microfluidic chip, capillary force was used to drive the fluid through the channels in the chip. In doing so, the fluid moved past a thin light sheet, created using an LED light and a microlens. The light sheet was adjusted at an angle, so that the light would cut through the flowing fluid without being pointed directly into the CMOS imager, which was placed below the microfluidic chip. When particles in the fluid flowed through the light sheet, light was reflected off the particles and into the CMOS imager. This signal was used as validation for the presence of particles in the sample fluid.

Though this research product had a fairly low detection time, the product was in need of a CMOS imager, which is fairly expensive and not simple to use. Furthermore, the design lacked portability due to the utilization of the CMOS imager. In seeing this, the design was noted as a great reference, as it was able to detect particles in sample fluid, but not fit for the application of detecting pathogen contamination in water for developing countries.

The next analyzed product was the *Watersafe Drinking Water Test Kit Bacteria* by *Filters Fast LLC* [8]. This consumer product provided 10 tests to find bacteria in water for $50.00. In order to complete a test, a small capsule was to be filled with sample water, which required 48 hours before the water in the capsule was able to be declared as contaminated or not based on the altered color of the water inside the capsule.

After analyzing this product, a few desirable characteristics were found. Since the only human interaction with the product was filling the capsule with sample water, the simplicity of this contamination detector was deemed one of its best assets. Furthermore, only a small capsule was needed for the detection to take place, causing no need for special equipment and allowing for a

portable way to detect bacteria in water. However, the product had a long detection time and an unreasonable price for people in developing countries. In seeing this, the simplicity and portability of the product were admired while the other characteristics were rejected for a product specifically designed for developing countries.

A last product that was used as a model for our senior design project was a research product designed to be a point-of-care diagnostic tool to detect Salmonella Typhimurium in water [9]. According to the research paper, the product was able to make its detection in less than a minute using a smartphone application. This product consisted of a paper microfluidic device that was loaded with anti-Salmonella conjugated sub-microparticles. Once the paper was dipped into a water sample, the smartphone application helped the user to place the phone at the necessary distance and angle from the microfluidic device in order for digital images to be taken and processed. In processing these images, the concentration of the sample was able to be calculated and displayed on the smartphone.

This research project was very similar to the design of our product. In analyzing the product, the contamination detector was found to be portable and have a low detection time. Also, the only special equipment needed for the detection process was a smartphone, which many people own in developing countries. The cost of this research product was also thought to be low, as there were no observable expensive parts used in the product. However, because the product uses a paper microfluidic device, the product would probably be susceptible to contamination in a rural environment. As a result, the product was remembered as a great model of what our product should become, with the exception of being a paper microfluidic chip.

In analyzing these products, models for the design of our product were obtained and desired aspects were noted. In the hopes of creating an *E. coli* detection system specifically for developing countries, the desired aspects that were reviewed will be considered when designing our product.

2.5 System Level Issues, Options, Trade-offs, Rational for Choices

After having an initial design for the project, some general system level issues, options, and tradeoffs were acknowledged. One of the biggest issues acknowledged in the design of the product was the lower detection limit of *E. coli* in the fluid samples. Since the product did not use high technology to detect bacteria, there was a possibility that *E. coli* would still be present in the fluid sample, even if the detection method said there was not, due to its inability to detect such small traces of *E. coli*. In seeing this, a tradeoff of whether the product should cost less, be portable, and be time efficient or have a lower detection limit was acknowledged. However, after reviewing the customer needs report, it was decided that low cost, portability and time efficiency trumped precision.

Another system level option was to decide which detection method to incorporate into the design. Though a few detection methods were thought of, only one was to be used in the final design. One of the contesting detection methods used optics to gain a signal from the cultures of latex particles that bound to the *E. coli* cells in the sample fluid. Another detection method measured the change in the electrical impedance of the sample fluid after the latex particles had bound to the *E. coli*. In doing this, the concentration of the *E. coli* should be able to be calculated from the change in impedance. A third detection method idea required multiple mixing chambers with different concentrations of latex particles in each. After the sample fluid flowed into each chamber and reacted with the latex particles, a saturation of the latex particles could be observed in one of the chambers, which could help to calculate the *E. coli* concentration of the sample fluid.

In thinking about these different detection methods, the customer needs report was again consulted and it was found that the detection method should follow the trend of low cost. All of the detection methods that were thought of—if implemented correctly—could be cheap to implement. Therefore, all detection methods were further analyzed with regards to other characteristics, such as detection time, ease of use, size, accuracy, human interaction, fabrication accuracy, and portability. This was completed in a selection matrix, which may be found in

Appendix D. After analyzing each detection method in the matrix, all four methods were found to be effective according to the matrix and the characteristics that were analyzed. In seeing this, all of these options for the detection method were kept in mind as the product progressed.

2.6 Layout of System Level Design with Subsystems

The design of the system was fairly simple as it consisted of only two parts: the microfluidic chip and the detection method. The microfluidic chip consisted of a small slide of glass and two thin layers of plastic tape stuck to the top of it. Microchannels were cut into the first layer of tape and small holes at the inlet and outlets of the channels were cut into the top layer of tape to provide a pressure difference and to allow sample fluid to flow through the channels. In doing so, the sample fluid could automatically move into a mixing chamber carved in the middle layer of tape, where the sample fluid could mix with latex particles. Based on the microfluidic chip testing results, a manual fluidic valve is used to keep the fluid within the chamber during mixing. Once the recommended time for latex particles to bind to *E. coli*—two minutes—has passed, the valve will release and allow the sample fluid to flow through the rest of the chip. It is at this time that the chosen detection method will be used for calculating the concentration of the fluid sample. This is how the subsystems work together to calculate the concentration of the sample fluid.

2.7 Team and Project Management

2.7.1 Project Challenges and Constraints

In thinking about the reality of the project, many challenges and constraints may be seen. One constraint that changed the scope of the project was realizing the minimum channel width for the microfluidic chip. Since vinyl cutters—the instrument used to cut the channels—have limited cutting widths due to the size of their blades, the channel widths were limited to approximately 0.1 mm. In seeing this, the automatic microfluidic valve was more difficult to functionalize, as we were unable to work in the nanoscale, where automatic valves are typically functional. This

obstacle provoked the consideration of utilizing a manual microvalve in the microfluidic chip, which would be controlled by the user of the device.

Also, in recognizing that the goal of the product was to accurately detect *E. coli*, there were a few risks that were necessary to take in the creating the product. One of these risks was interacting with *E. coli*, which could be caught by a team member in completing a biohazardous test. Another potential danger in this project was the glass that was used in testing. The glass slides were very thin and capable of shattering into many pieces, which could lead to injury if not cleaned up properly. Therefore, extra care was taken to not drop or break a glass slide.

2.7.2 Budget

2.7.2.1 Hydrophilic Adhesives

The ARFLOW 93049 hydrophilic adhesive used was provided by Adhesives Research free of charge for research purposes. Therefore, for the purpose of developing this product, there was no cost for using this material. However, the actual price of the hydrophilic adhesive was tabulated into the total cost of the finished product.

2.7.2.2 Graphtec CE6000 Cutter

In order to make precise cuts, a Graphtec CE6000 cutting plotter was purchased at a price of $1100.00. The cutter was a one-time cost that was expected to perform all cutting required for the project. The only maintenance cost was buying new cutting blades when the blade dulled. A set of two cutting blades costs $77.00.

2.7.2.3 *E. coli* Detection Kit

The *E. coli* detection kit with latex particles yielded the greatest material cost for the project. Each detection kit cost $120.00 and was designed to provide fifty detection tests. We planned to use three of these detection kits. Part of our project included experiments to measure the effect of varying latex particle concentrations to see if less latex particles than the recommended amount could be used to effectively perform the detection tests. This would not only reduce the cost of our project, but also reduce the cost of the final product.

2.7.2.4 Glass Slides

Two hundred glass slides were purchased at a price of $0.10 per slide with a total cost of $20.00. Glass slides should not be reused due to contamination, which justifies the quantity purchased.

2.7.3 Timeline

In order for the project to run smoothly, a timeline was created to gauge when certain parts of the project should be completed. The project taken on was an 8 month project that was expected to be finished by May 2016. In the Fall of 2015, the major goals were to characterize the fluid flow within the hydrophilic adhesive channels and develop an appropriate flow control mechanism. One possibility for this mechanism was a capillary burst valve, which would stop the flow in the mixing chamber until the sample fluid from a second channel released it. Though this was an important aspect of the microfluidic chip, the very first task was to determine which pathogen to detect, as this information was crucial to how the rest of the research and experiments unfolded. After, the flow speeds and geometries for the chosen pathogen to detect could be optimized and the fluid flow could be characterized.

In the Winter of 2016, the goal was to develop and implement an appropriate detection method. There were several possibilities, but using a lens to gain a signal of the particles flowing through the channels seemed the most promising. Another possibility was to measure the electrical impedance of the flowing fluid. Since the goal was to create a simple, user-friendly system that affected the greatest amount of people, a computer or smartphone application was planned to be written in order to easily interpret the data and calculate the contamination.

In the Spring of 2016, the product development was finished, giving ample time to work on presenting the findings from the project in presentation and thesis form. The presentation has been finished and the thesis is complete. The plan for each quarter is shown in the Gantt charts in Appendix D.

2.7.4 Design Process

In designing the product, many different options were brainstormed and the most plausible ones were further explored. Initially, for the microfluidic chip, two options were considered: a paper microfluidic chip and a gravity powered microfluidic chip. In researching the paper microfluidic chip, it was found that a capillary flow was guided within the paper by hydrophobic wax to drive the fluid. However, in considering the paper microfluidic chip, it was acknowledged that the opacity of the paper would disallow the use of any optical methods of detection. Since optical detection was one of the more promising detection methods, this type of microfluidic chip was less desirable for the product. In the gravity-assisted microfluidic chip, a plastic chip would be oriented at an angle in order to use gravity as a driving force instead of an external pump. Because it could be created on transparent material, the gravity-assisted microfluidic chip could be used with an optical method of detection; however, control of the flow may have been difficult due to the constant driving force of gravity, meaning that the driving force could not be stopped. In seeing that neither of these options provided all of the desired characteristics for the microfluidic chip, a method combining the desired characteristics from both methods was created.

In this newly created method, it was decided that a platform using hydrophilic adhesive would be best, due to the ease in manufacturing and the ability to provide a driving force without additional energy sources or pumps. In designing the valves on the new material, valve designs were researched in scholarly journals and imitated as closely as possible to analyze their functionality. An iterative process was implemented to change certain parameters—mainly geometry—of the valve in order to functionalize it. Also, multiple hydrophilic tapes were tested to see what effects the hydrophilic properties had on the flow rate and valve functionality. Because the valve was not consistent in actuating the flow, a manual valve was tested. While this did require one more user input, it improved the consistency of the product.

In the design process of the detection method, the team started out with three different ideas: optical detection, electrical impedance detection, and multiple chamber analysis detection. Since time was limited and only one detection method was necessary for the product, a quick analysis

of all three detection methods was made and it was found that the optical detection method was the most fitting for *E. coli* detection in developing countries. In functionalizing this detection method, it was recognized that there were three necessary parts: a microlens to see subtle signals from an analyzed fluid sample, a camera for collecting the signals, and an algorithm to quantify the signals. To create such a detection method, the initial step was to acquire a lens and a camera. In gaining these items, preliminary images of what the algorithm would be analyzing could be obtained. These images would be used to develop the algorithm used to find the concentration of *E. coli* in the fluid sample. Initially, a low cost microscope was used with a smartphone camera to test out a range of magnifications and how they would look on the smartphone. These were the initial steps in the design process of the detection method.

2.7.5 Risks and Mitigations

In thinking about the risks present in the fabrication, testing, and disposal of our product, a few safety concerns should be discussed. In the manufacturing process of the product, a vinyl cutter was required, as this machine could obtain the necessary precision to make functioning microfluidic chips consistently. Within the cutter, a sharp blade moved autonomously according to the designed cut and could only be stopped by pressing the pause button on the machine. While the cutting area of the blade was small and covered, it was safe practice to avoid putting hands or other body parts near the blade when it was in operation. In addition, it was safe practice to keep clothes, hair, or other objects that may become caught away from the moving machinery in the cutter. Failure to do so could result in an individual being pulled into the cutter, which could have caused minor injuries.

In assembling prototypes, glass slides were used for the bottom layer of the microfluidic chip. While the glass slides were not sharp, dropping one could have caused it to shatter into sharp parts. For this reason, glass slides were kept away from table ledges whenever possible and were handled with care. In the event that glass did break, the broken pieces were cleaned up immediately with a broom or brush and dustpan, and then disposed of in the nearest broken glass disposal.

Exacto knives were also used in the fabrication process to peel the channels from their adhesive cover. The knives used were not very sharp; however, common knife safety was used whenever handling a knife. This meant no pointing the knives at others and always cutting away from any individuals. Also, if the blade of the knife was retractable, it was retracted whenever not in use.

In the testing phase of our senior design project, similar safety precautions were carried out for testing of the microfluidic chip, but more precautionary actions were implemented when testing the detection methods. In order to run real detection tests of the product, non-pathogenic, heat inactivated *E. coli* O157:H7 cells were used. While the particles posed no threat to human beings, they were properly disposed of in a biohazardous waste bin. While performing the tests for *E. coli*, proper biohazardous safety equipment (e.g. a mask and gloves) was used to mitigate the chance of coming into contact with dangerous pathogens. Once the tests were complete, the glass slides and testing supplies were stored in appropriate areas. This meant the storage area was not out in the open or on high shelves, where a glass slide could fall and shatter.

Another device that needed to be stored properly was the cutter. This expensive piece of equipment was kept in an area where it could not be damaged and where others would have limited access. Since most of the equipment used for testing the different designs was from Santa Clara University's Biology Lab, the equipment was cleaned after testing and replaced where it was found. In doing so, not only was equipment prevented from being lost, but others were able to use the equipment when it was not being used for our senior design project.

In disposing the materials used for testing the product, it was important that each material was placed in the proper disposal container. When testing the microfluidic chip, the materials used were not considered to be hazardous; however, if glass were broken in one of the tests, the glass needed to be swept up and placed into a glass disposal container. The adhesive and tape used for the top layer was able to be disposed of in a regular garbage disposal. In the tests using the positive for the *E. coli* contamination, the entire system was disposed of in a biohazardous disposal or sterilized before disposal. In order to sterilize the systems, an alcohol bath, boiling, or bleach was used to remove microbes. This ensured safety for the experimenters when disposing of the different materials in testing the product.

2.7.6 Team Management

From the start of the project, team members quickly moved into positions where they felt comfortable and were able to use their strengths to help advance the project. Each member was able to carry his own weight, and in times of struggle, others were able to step in and help out to continue the advancement of the project. Each week, the team members were able to meet twice: once to create test fixtures and another time to run tests and record the results. A third meeting was made every week with the senior design advisors in order to present the results, compare the accomplishments with goals created in the prior meeting, and create new goals for the following week. In this meeting, results and the next steps of the project were discussed while insight was given from the team advisors.

The management carried throughout the year consisted of each person wearing a couple of different hats. For example, in meetings, Andy and Andres took the role of a scribe, as they wrote down the goals, ideas, suggestions, and tasks for each team member at the meetings held with the project advisors. In doing so, they were able to help structure the team and allow for the advancement of the project by assigning weekly tasks to all members of the team. Also at the meetings, each member typically presented the findings of the experiments they had completed during the previous week. Afterwards, all team members provided insight to the results of the experiments. However, these were not the only jobs of the team members throughout the year. Andres was also involved in analyzing the data obtained from the experiments that were carried out and making correlations between flow rates, channel widths, detection limits, and channel materials so that desired results could be obtained in the next test. Alternatively, Andy was involved in researching preexisting information about the different aspects of our product and provided assistance to Scott, as Andy had the most bioengineering knowledge out of the three mechanical engineers. Being the only bioengineer on the team with a substantial amount of time in a laboratory setting, Scott was assigned most of the work that involved diluting the *E. coli* and analyzing the binding abilities of the latex particles. Kyle was assigned most of the design work for the microfluidic chips, due to his past experiences in SolidWorks, and most of the work for writing and testing the detection algorithm. Lastly, all members took part in the experimental

work of fabricating the microfluidic chips and analyzing the test trials of the integrated detection system.

In trying to decipher the different roles each member held in the team, one must remember there was a similar amount of work contributed by each member. In seeing this, one should come to the conclusion that no single member had been deemed the leader of the team, as the team worked better without one. Due to the work ethic of all members on the team and the team's ability to strategically divide the work depending on each member's strengths, the team's progress was able to increase without any need for a leader to check the progress of the team and push the team to continue working on the project. Therefore, based on the similar personality types of the team members, the team was able to make it quite far without a chosen leader.

2.8 Building and Buying Plan

In building and buying the materials and hardware necessary for the project, there were only a few delays to the project's progress. As far as materials, adhesive plastic obtained free of charge to use for creating microchannels and glass slides were obtained rather quickly through internet sources. In using these materials, an initial microfluidic chip was able to be made with flat, concealed microchannels for the sample fluid to flow through. Since our product was so simple, limited hardware was necessary to build, test, and prepare the final product. To cut the microchannels into the adhesive material, a vinyl cutter was used, which was the only hardware necessary for building the microfluidic chip. This piece of hardware was bought with the money allocated by Santa Clara University's School of Engineering and acquired early on. Therefore, there were no problems with the lead times or high cost. Also, for the few weeks that the cutter was not available, a different cutter, available in Santa Clara University's Maker Lab, was used.

Other pieces of equipment necessary for the completion of the senior design project were a microscope, microlens, and smartphone. These pieces of equipment were to be used in testing the detection methods of the project and were very easy to acquire. A low cost microscope was able to be bought through internet sources and arrived at the school rather quickly. Likewise, the microlenses that were used in testing the detection method were purchased online and mailed to

our lab. These pieces of equipment were paid for with the project funding from the School of Engineering. As for the smartphone, all members on the team owned a smartphone and were able to use it when necessary for the project.

During the beginning of Spring quarter, the team ran out of *E. coli* for testing the fully integrated system and needed to purchase more in order finish the product. In doing so, the *E. coli* order was backed up, causing a four-week delay time on the project. During this time, the members found other aspect of the project to work on; however, this was a major inconvenience in completing the project.

Chapter 3: The Microfluidic Chip

3.1 Introduction

In designing the microfluidic chip, many factors affecting the functionality were considered to create a chip capable of performing the three following desired functions:

- Driving the fluid
- Mixing the fluid
- Providing an area to utilize a detection method

The microfluidic chip must facilitate mixing between a fluid sample and latex particles so that the latex particles can combine with the *E. coli* and form agglutinates. Agglutination in the fluid sample is important because it makes the *E. coli* cells much easier to be detected with a detection method. Additionally, the microfluidic chip must be able to move the fluid from one point to another and have a section where a detection method can make a calculation of the *E. coli* concentration in the fluid sample. Lastly, the microfluidic chip must meet the ASSURED standard as closely as possible to be effective for use in developing communities.

3.2 Options and Trades

In thinking about how the flow may be driven with the microfluidic chip, a few methods were brainstormed and analyzed. This ultimately allowed for the advantages and disadvantages of each method to be revealed. The following four options for driving the flow were considered and analyzed in designing the microfluidic chip:

- **Capillary-Driven Flow:** This method uses the surface tension at the interface between the fluid and a hydrophilic material to move the fluid through the chip. It requires no external equipment, as the fluid-driving capabilities are inherent in the properties of the

material used. However, this method is limited in its ability to control the speed of the fluid flow because it does not have the level of control that other methods might have.

- **Gravitational Flow:** This method requires the microfluidic chip to be placed at an angle such that the gravitational forces can pull the fluid downward. The slant angle of the chip and the geometry of the channels in the chip can be designed to affect the speed of the flow at different points within the channels. This method requires no external equipment, which adds to its simplicity and reduces its cost. However, the direction of the fluid flow is constrained by the fact that the fluid can only flow downward. Additionally, the geometry of the entire *E. coli* detection system apparatus is constrained by the required angular placement of the chip.

- **External Pumping:** This method is quite straightforward, as an external pumping device would be used to create enough pressure to drive the fluid through the channels. The pump may be powered electrically or manually and has the potential to provide a relatively high level of control over the fluid flow. However, the pump will require external equipment and power which add to the complexity and cost of the microfluidic device.

- **Centripetal Force-Driven Flow:** In this method, the chip is spun around in an external piece of equipment, causing centripetal force to pull the fluid away from the center axis of rotation. The axis of rotation would be parallel to the surface area vector of the microfluidic chip. This method allows a relatively high level of control over the fluid flow, but the direction of the flow is limited. Additionally, this method requires external equipment and external power sources, which add to the complexity and cost of the system.

A summary of each method and their trade-offs are presented in Table 3.1. In our chip, the capillary-driven flow method was selected to drive the fluid, as it followed the ASSURED standard by being simple and cheap to implement.

Table 3.1: Summary of options for driving the fluid in the microfluidic chip.

Option	**Description**	**Advantages**	**Disadvantages**
Capillary-Driven Flow	Use capillary forces to move the fluid with the surface tension of the contact between the fluid and a hydrophilic material.	• Fluid driving built into the material • No external power sources or equipment necessary • Low cost	• Control of flow speed is limited
Gravitational Flow	Use gravitational forces to move the fluid by having the fluid flow down an incline.	• No external power sources or equipment necessary • Low cost	• Chip geometry is constrained • Direction of flow is limited by force direction
External Pumping	Use an external pump to move the fluid.	• Fluid flow speed can be controlled	• External power sources and equipment are required
Centripetal Force-Driven Flow	Use centripetal force to move the fluid by spinning the chip.	• Fluid flow speed can be controlled	• External power sources and equipment are required • Direction of flow is limited by force direction

After analyzing how the sample fluid would move through the microfluidic chip, methods of mixing the fluids in the chip were analyzed. In brainstorming different methods, three plausible options were considered, as listed below:

- **Diffusion Chamber Mixing:** This method utilizes the diffusive properties of fluids to mix a number of fluids in a mixing chamber made directly in the microfluidic chip. When the fluid sample comes into contact with the latex particles in the mixing chamber, the

fluids will slowly mix with each other until they reach equilibrium. The amount of mixing time required for this process can vary, but in small quantities, the mixing time can be less than 10 minutes. This method requires a chamber for mixing, but requires no external equipment to facilitate the mixing. Additionally, this method mixes autonomously and the design is not expensive to implement.

- **Manual-Mechanical Mixing:** This method utilizes manually mixing of the fluid sample by the user. Methods of manually mixing the fluids include, but are not limited to, stirring or shaking the sample. The mixing time for this method is low and mixing can be done in a chamber on the chip or in a separate surface or container off the chip.

- **Automatic-Mechanical Mixing:** This method utilizes an automatic system, such as a mechanical stirring chamber, to mechanically mix the fluid. The mixing time for this method is low and the system is automated, but the system requires external power and can be very complex.

After analyzing each option for mixing the fluids in the microfluidic chip, the diffusion chamber mixing method was found to be the best fit for our device. This is because it required no external equipment and allowed for automated mixing. Also, the time required to mix the fluid was short enough by our standards to still be considered “rapid”. The following table summarizes the mixing methods that were considered.

Table 3.2: Summary of options for mixing the fluid in the chip.

Option	Description	Advantages	Disadvantages
Diffusion Chamber Mixing	Use diffusion to mix fluids in a mixing chamber directly fabricated on the microfluidic chip.	• Low cost • No external power sources or equipment necessary • Mixing is done autonomously	• Mixing is slower than other methods
Manual-Mechanical Mixing	Use manual mechanical mixing from the user (stirring or shaking the fluid).	• Quick, thorough mixing	• Requires additional user interaction
Automatic-Mechanical Mixing	Use automatic mechanical mixing from a device (stirring or shaking the fluid).	• Quick, thorough mixing • Mixing is done autonomously	• External power sources and equipment necessary

Lastly, in thinking about the static or dynamic state of the fluid sample as it is analyzed by a detection method, two options were considered:

- **Static State:** This method involves having the fluid sit statically in a chamber after it finishes mixing. A detection method would then analyze the detection chamber and calculate the concentration of *E. coli* in the fluid sample. The implementation of a static section design is simple, but the amount of sample that is being used to provide results on a larger quantity of fluid is small. This makes it necessary that the detection system analyzes as much area as possible in the detection chamber to receive a reasonable sample size.

- **Dynamic State:** This method involves having the fluid flow through a detection channel where the detection system can take measurements on the flowing sample. This system reduces the complexity of the detection method, as a larger fluid sample can be analyzed by flowing it through the detection method than when the sample is stationary. This system is mildly complex and requires some channel analysis to make feasible.

In comparing the two fluid states, the dynamic flow detection was selected because it required a smaller area for analysis and was able to analyze a greater quantity of fluid. The following table summarizes the two fluid states for the detection method.

Table 3.3: Summary of options for fluid detection states.

Option	Description	Advantages	Disadvantages
Static State	Fluid is analyzed in a chamber where fluid is static.	• Design is simple	• Detection area is limited
Dynamic State	Fluid is analyzed in a detection channel where the fluid is flowing.	• More fluid is analyzed	• Channels must be designed for certain flow conditions

3.3 Design Description

Based on the design options selected, it was decided that a hydrophilic adhesive tape would be used to drive the fluid through the chip. This material was easy to use, as channels, inlets, and outlets could be cut into the tape. These geometries were then able to be placed on a flat material to create the chip.

In order to create and test variations of the microfluidic chip, a vinyl cutter was used to cut channel geometries into the hydrophilic adhesive. In using this machine, repeatability and a high

amount precision was obtained in the cuts made for the channels. The basic design of the microfluidic chip geometry consisted of an inlet, an incubation chamber, a microfluidic valve, and an outlet. Figure 3.1 displays the different parts of the geometry in the microfluidic chip.

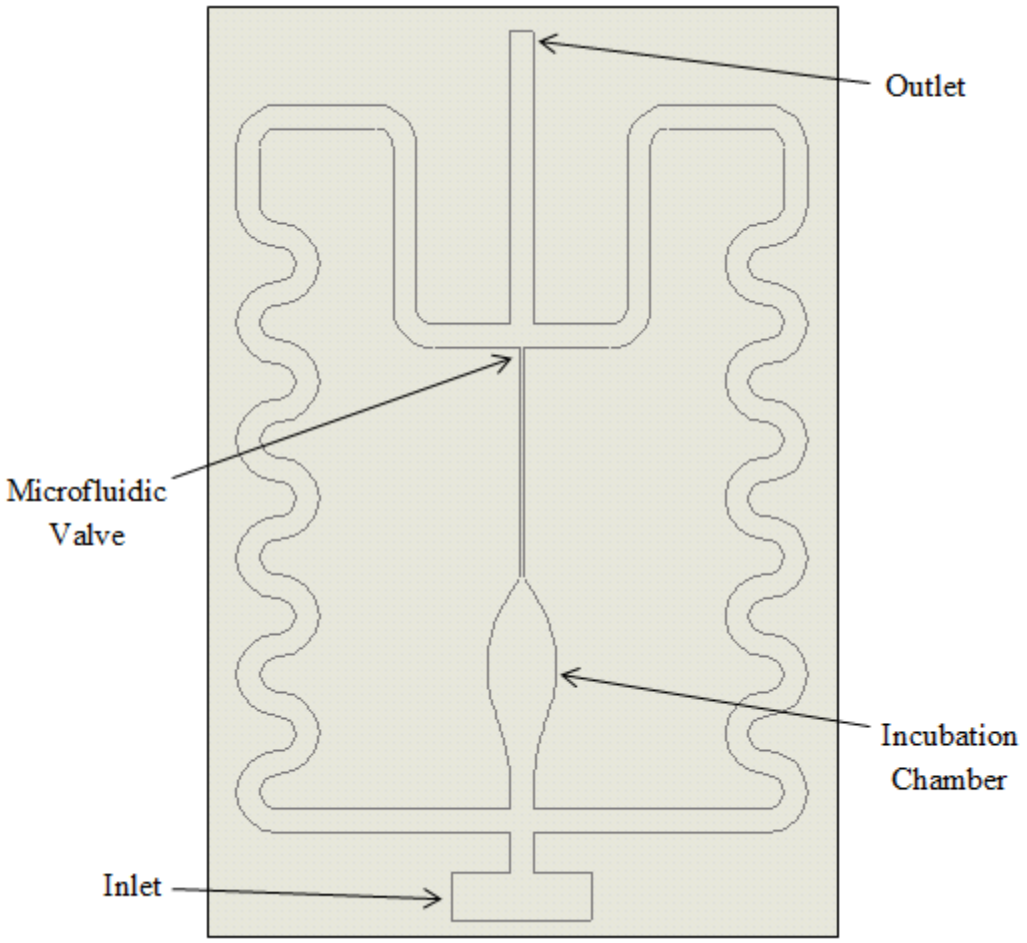

Figure 3.1: The basic geometry of a microfluidic chip.

As shown in Figure 3.1, the design of the chip was simple enough to use as little material as possible while still accomplishing its main tasks. The geometry of the chip's inlet was not the most critical part of the chip, but having an adequate space for the sample fluid to sit while being fed into the channels of the chip was a desired asset. Having this space helped to contain the sample fluid at the inlet and keep the user from touching the sample fluid.

In designing the incubation chamber, the width was adjusted to allow enough area for the sample fluid to agglutinate with the latex particles. Increasing the width of the chamber allowed more sample fluid to enter and incubate with the latex cells. The shape of the chamber was arbitrary, as the chamber needed to take fluid from a thin channel, increase the width to create an incubation area, and then decrease the width into a very thin channel to allow for a microfluidic valve to stop the fluid from leaving the chamber. This is the reason for the chamber's shape in Figure 3.1.

The tested designs varied mainly in channel widths, as changing this parameter was the most effective way of altering the flow rate through the microfluidic chip. In altering the channel widths, the Washburn equation, shown below, was used to calculate the distance the fluid traveled at certain points in time [9]:

$$L^2(t) = \left[\left(1 - 0.63\frac{h}{w}\right)\frac{h\gamma\cos\theta}{3\mu}\right]t \quad (1)$$

where L is the distance traveled by the fluid, h is the height of the channel, w is the width of the channel, Θ is the contact angle, γ is the surface tension of the fluid, μ is the viscosity of the fluid, and t is the time since the fluid was placed into the inlet. From this equation, the velocity of the flow through the channels in the chip could be calculated prior to designing and building the microfluidic chips. The most space and material efficient way to incorporate this incubation time was thought to be by making multiple turns in the timing channels to keep the design compact. Lastly, the number of timing channels created for the fluid to flow through was altered in order to see if more channels would help relieve the pressure that would presumably build up at the incubation chamber and microfluidic valve.

The last design aspect in the channels of the microfluidic chip was the outlet geometry. Like the inlet, this aspect of the microfluidic channel geometry was not thought to be of much importance, as altering it would have a negligible effect on the flow of the sample fluid. However, having a larger outlet would provide a place for the fluid to flow into after flowing through the channels in the microfluidic chip. Since these five aspects were thought to have the greatest effect on the

flow of the sample fluid through the microfluidic chip, they were deemed the main varying parameters of the chip.

3.4 Drawings

The drawings depicted below are a few of the important versions of the microfluidic valves that were tested in our project.

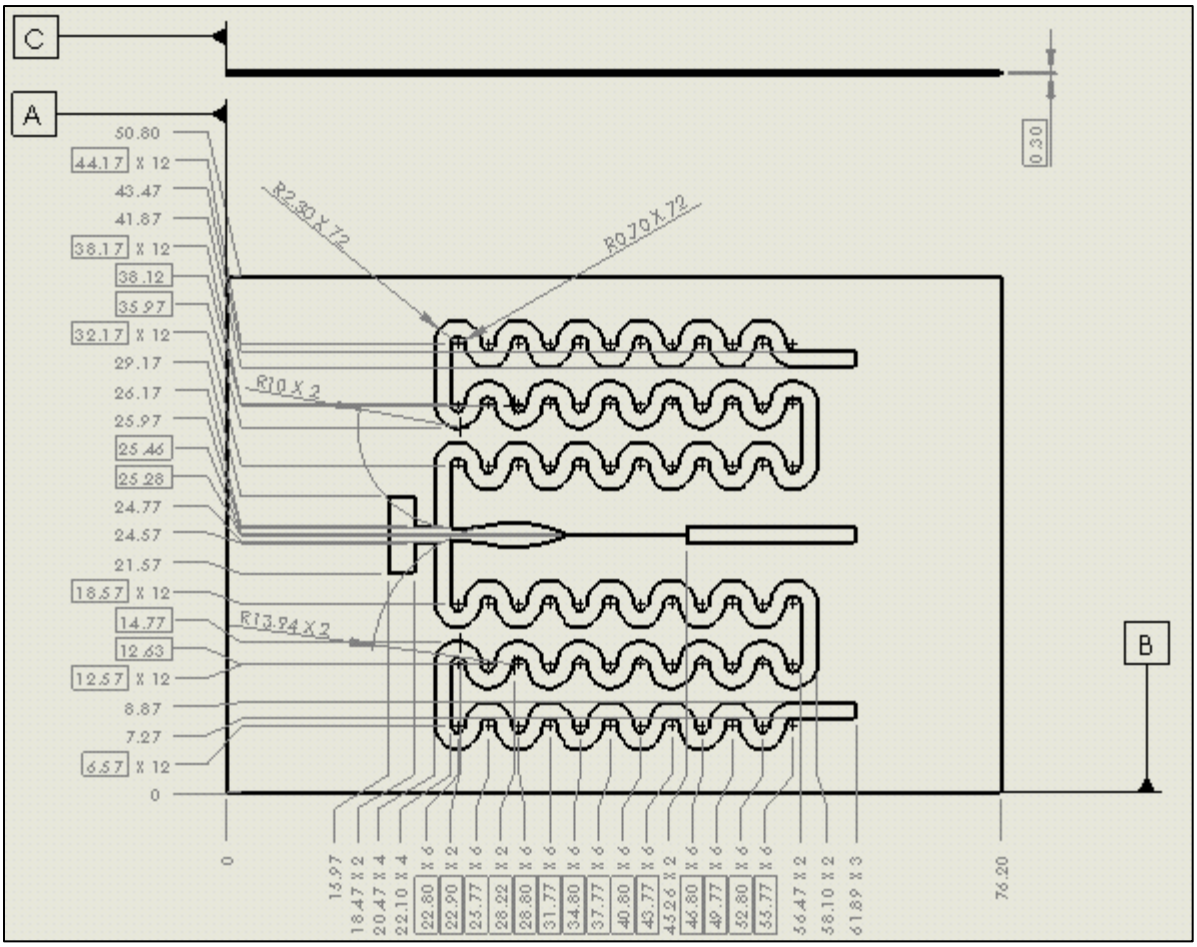

Figure 3.2: The drawing of the middle layer of the automatic microfluidic chip. All dimensions are in millimeters.

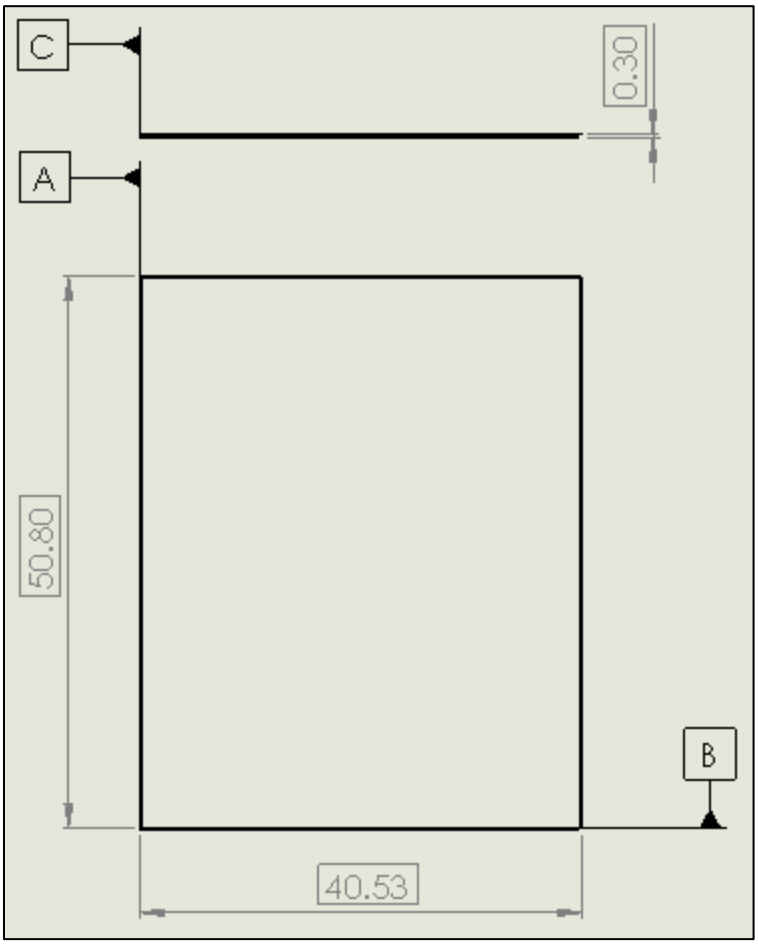

Figure 3.3: A drawing of the top layer of the automatic microfluidic chip configuration. All dimensions are in millimeters.

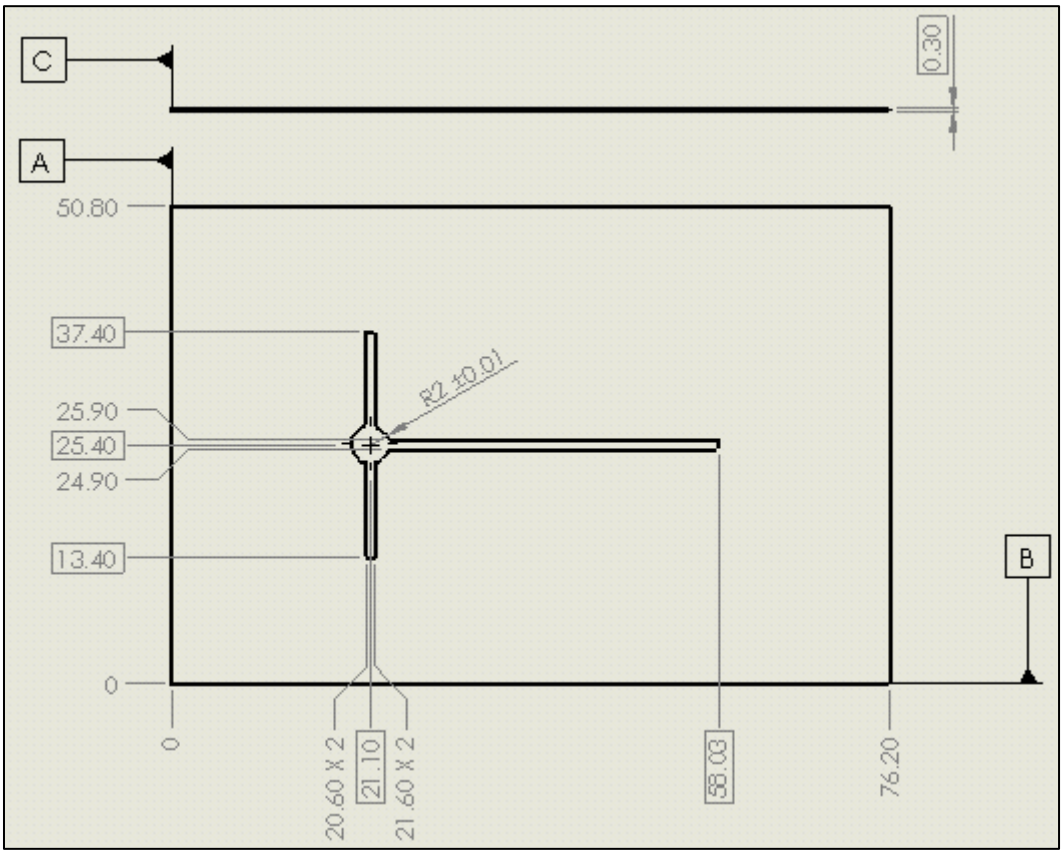

Figure 3.4: A drawing of the middle layer of one of the manual microfluidic chip configurations. All dimensions are in millimeters.

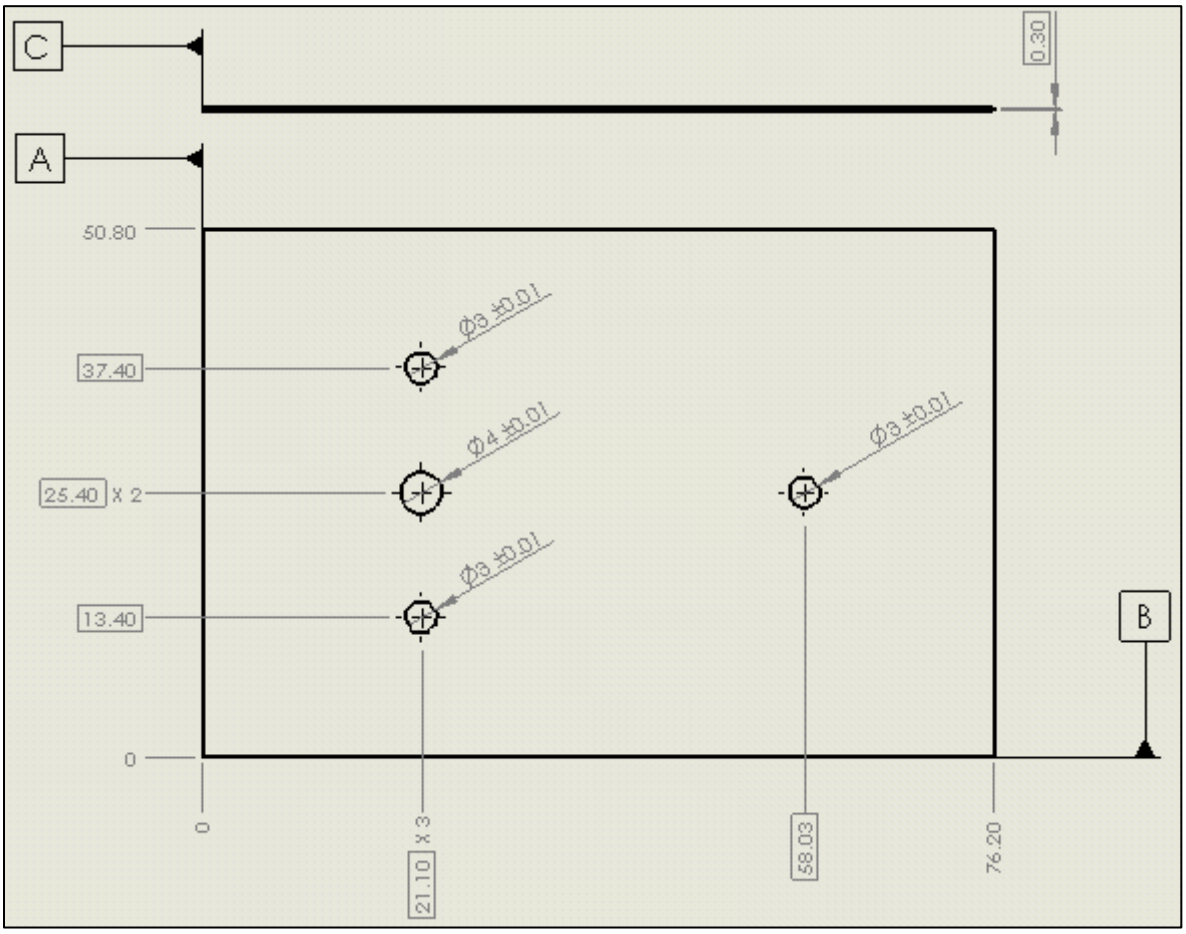

Figure 3.5: A drawing of the top layer of one of the manual microfluidic chip configuration. All dimensions are in millimeters.

3.5 Materials and Methods

In the fabrication process of the microfluidic chip, multiple materials were used in attempting to create a chip that would allow the sample fluid to incubate with latex particles and send the formed agglutinates to a detection channel. The materials used in creating the chip could be placed into two categories: hydrophilic and hydrophobic materials. Materials that were hydrophilic had a tendency to spread any fluid it came into contact with along its surface. Alternatively, hydrophobic materials had a tendency to keep a fluid in a contained area and not allow it to spread over its surface. In seeing this, materials of the two categories were utilized in order to create a valve that could hold back the fluid in the mixing chamber until it was time to let the fluid out. To determine whether a material was hydrophilic or hydrophobic, the contact

angle that the material made with the sample fluid needed to be measured. This could easily be completed by placing a drop of the sample fluid onto the material being analyzed and then measuring the angle between the surface of the fluid and the material, as shown in Figure 3.6.

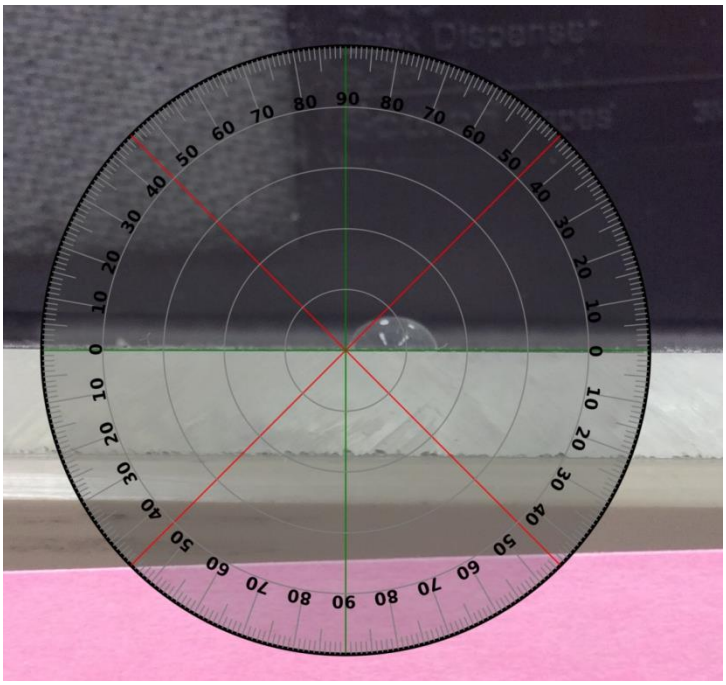

Figure 3.6: An experiment to determine the contact angle of acrylic. The angle may be determined from the superimposed protractor.

In seeing the importance of the material used in the valve, the materials of all three layers of the microfluidic chip were altered to see the effects it would have on the valve. For the top layer, a microfluidic adhesive tape was initially used, as it was specifically made for the application at hand. Since this material was known to be very hydrophilic, other materials that were less hydrophilic, such as different types of tapes, were planned to be tested in case the hydrophilic tape did not function properly. As for the middle layer, the microfluidic adhesive was initially used. This was mainly due to the fact that the tape was easy to carve channels into using a vinyl cutter (Graphtec CE6000), as the middle layer was the layer with the channels. Lastly, the bottom layer was initially a glass slide, in order to give the product a rigid support to operate on. However, since glass was found to be very hydrophilic, acrylic was also planned to be tested as the bottom layer material, as it was less hydrophilic than glass.

As far as methods for creating the test chips, there was only one process considered, as it was very straightforward and allowed for high precision in the geometry of the microfluidic chip. To start this method, channels for the microfluidic chip were designed using SolidWorks to create precise dimensions and intricate geometries. These drawings were then loaded onto a vinyl cutter, which carved the designs into the hydrophilic adhesive tape being used as the middle layer of the chip. It is important to note that the carvings did not actually remove any material from the tape; they simply outlined the designs created in SolidWorks onto the tape. The tape, with channels now carved into it, was then placed onto a glass slide, which was used as the bottom layer. At this time, the material outlined by the carvings was peeled off the glass slide using a sharp edge. In doing this, the channels were created in the middle layer of the chip.

After, the top layer was created by cutting a piece of tape to the correct size, which would cover all channels without blocking the inlets or outlets. When applying the top layer of tape onto the middle layer, it was important not to push on the portions of tape above the channels in the middle layer, as the tape would stick to the bottom layer through the channels. This would create an obstruction for the sample fluid in the channels.

Finally, with the chip completed, the sample fluid was placed in the inlets with a pipette, starting the trial test of the microfluidic chip. At this time, it was usually desired to record the test with a phone video camera for later analysis on the flow in the channels. The process stated usually took less than 10 minutes, excluding the time to design the channels in SolidWorks.

3.6 Expected Results

Prior to completing test trials of flowing fluid through the microfluidic chip, some predictions were made on the behavior of the fluid when it was placed at the inlet of the microfluidic chip. Initially, when an automatic valve was implemented, the flow was predicted to fill the mixing chamber and stop at the intersection of the mixing chamber and the side of the crossing channel. It would not be until after the flow from the crossing channel reached the mixing chamber outlet that the fluid inside the chamber would continue to flow through the chip. It was also predicted that the timing for the fluid to flow through the entire chip would be very close to what was

predicted by the Washburn equation, based on the width of the channels in the chip [9]. Since the wide, hydrophilic adhesive tape was used for the top layer, as well as the middle layer, it was thought that the chip would have no leaks and have a relatively fast flow rate. In making these predictions the test trials were able to be carried out with a pre-existing sense of the results.

3.7 Backup Plan

In learning from prior research that the automatic microfluidic value was a difficult feature to implement from prior research, a backup plan was devised in the case our team was unable to create a functioning valve. In the process of testing different valve geometries and top layer materials, our team learned that the manual microfluidic valve was incredibly reliable and worked almost every time. In seeing this, our team agreed that if the automatic valve was non-functional, we could simplify the design of the microfluidic chip and use the manual value. Although this would add one more step to the detection process and more user interaction, it was deemed that a highly reliable chip was better than one with less user interaction.

3.8 Prototyping Results

In testing our microfluidic valve designs, insight that changed the scope of our project was gained. The most important result from our prototype testing was our automatic capillary burst valve designs failed to function consistently. With further analysis, the cause of this failure was found to be the scale of the system, as it was simply too large for the surface tension of the fluid to dominate in such a manner as to stop the fluid flow. The nanoscale is typically necessary to build a functioning capillary burst valve; however, we were only able to access the microscale due to the precision limitation of the cutter we used to build the channels. Since the automatic capillary burst valve designs failed, we resorted to our backup plan, which was to implement a manual valve in place of the automatic valve. After carrying out another iterative design process, the manual valve designs were found to work consistently and effectively for our purposes.

As for the chip geometry, all channel designs were found to work as planned. In earlier revisions of the manual microfluidic chip, the channel following the mixing chamber was a straight channel. However, this channel geometry caused the fluid to take only 2.5 seconds to reach the outlet after the valve had been activated. Since this was believed to be too short of a time frame for the user to implement the detection method after releasing the valve, the channel was redesigned in order to allow for a longer time frame for the user. To make the time frame longer, the channel length was increased by wrapping the channel a few times in a switch-back formation, as shown in Figure 3.7. The channel width was also decreased, as the Washburn equation demonstrated that a small channel width would slow down the flow. This can also be seen in Figure 3.7. Figures 3.7 and 3.8 show the final geometric configurations for the manual valve.

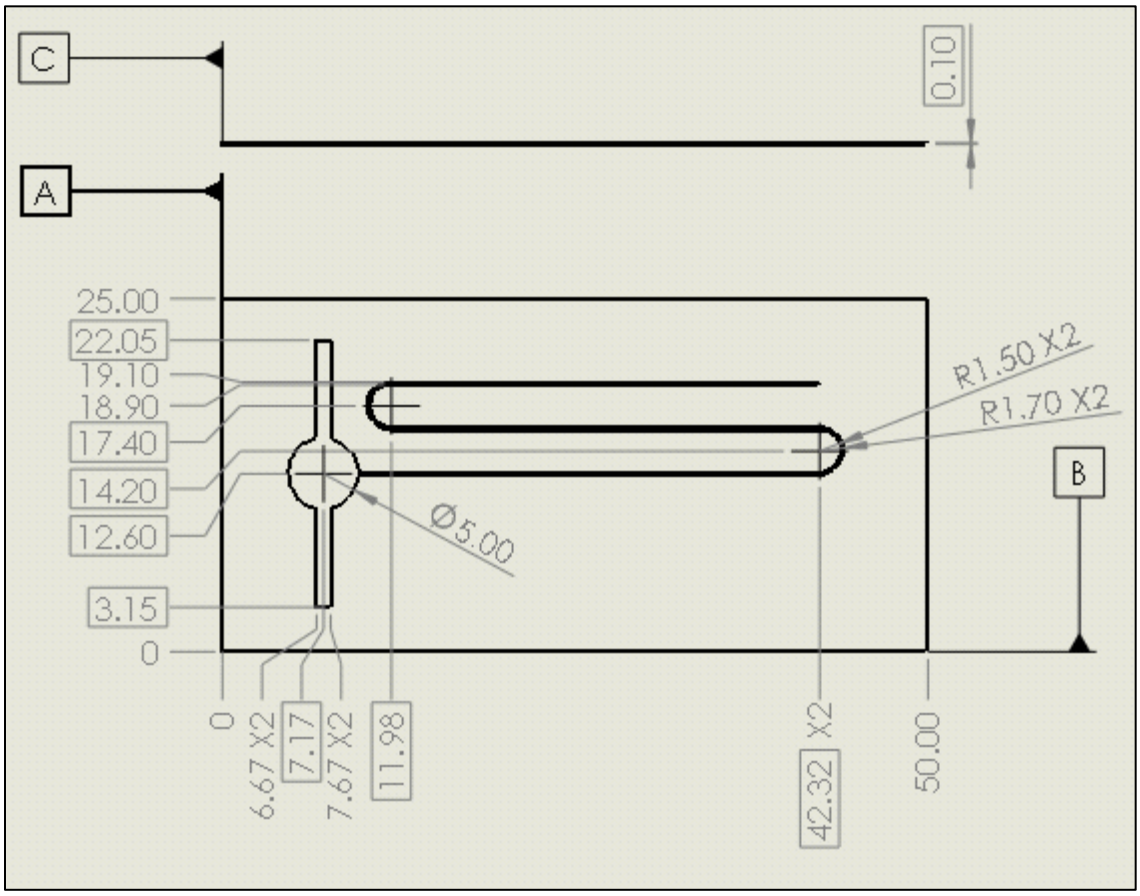

Figure 3.7: A drawing of the middle layer of the final manual microfluidic chip configuration. All dimensions are in millimeters.

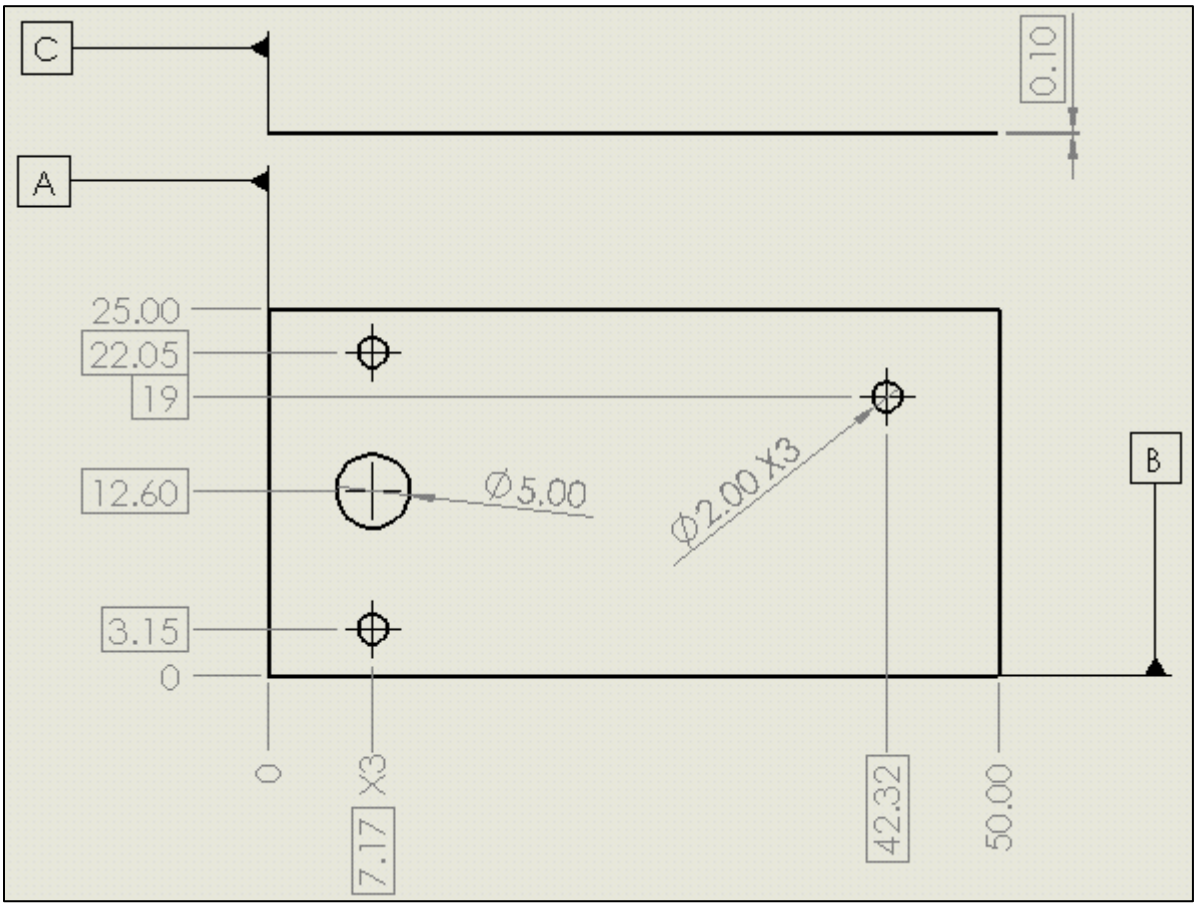

Figure 3.8: A drawing of the top layer of the final manual microfluidic chip configuration shown. All dimensions are in millimeters.

As seen in Figure 3.7 and Figure 3.8, the final manual valve design was very simple to manufacture, as a single chip took less than a minute to fabricate. As the manual valve was consistent in function and easy to fabricate, the project was able to progress with a strong foundation in its microfluidic chip component.

3.9 Results Analysis

In completing the test trials of the microfluidic chip and making a few modifications, some interesting results were found. Unfortunately, a consistent automatic valve was found to be irreproducible due to the scale of the system. However, the manual valve, which was initially a backup plan, was able to be functionalized and provided a reliable platform for the rest of the

detection product to be based upon. In implementing the manual valve, more user input was necessary for the bacterial detection to take place, but the consistency provided by the manual valve was agreed to be worth the small increase in user input.

After finishing the iterative process for finalizing the manual microfluidic chip, it was found that the length of the main channel in the chip was to be increased and the width of the channel was to be decreased. In completing these tasks, the finalized microfluidic chip was created and the detection method could begin to be produced.

Chapter 4: Detection Method

4.1 Introduction

The purpose of the detection component in our system is to detect *E. coli* contamination in the fluid sample on the microfluidic chip. In order to make this component more effective for use in developing communities, we used the ASSURED standard to guide our design.

As a starting point for our design, we decided to utilize latex agglutination to amplify the detection signal of the *E. coli*. Traditionally, a latex agglutination test consists of a static fluid sample mixed with latex particle and the user's discretion of whether or not agglutinates formed in the fluid. This method of detecting *E. coli* is quite qualitative and subjective. In order to more closely follow the ASSURED standard, we required our detection system to be able to not only detect *E. coli* concentration, but also output a concentration quantity. Because of this, our design was intended to build up from and improve the traditional latex agglutination detection method so that a quantitative concentration value could be obtained for a fluid sample.

As stated by the National Water Program, "the acceptable risk level for total body contact recreation, which involves activities such as swimming or water skiing, is 126 colonies of organisms (referred to as colony forming units or CFU) per 100 milliliters (mL) of water or less based on a geometric mean (calculated over 30 days with at least 5 samples) or a one-time concentration of 235 CFU/100 mL. The risk of getting sick increases as total numbers of colonies are exceeded" [10]. However, the standard for allowable *E. coli* concentration in drinking water is 0 colonies and up to 575 CFU/mL for activities with only partial water contact such as fishing. Traditionally, *E. coli* testing requires a sterile environment and expensive equipment, requiring five days to obtain results [11]. The latex agglutination method on which we based our detection method has a detection limit of 10^5 CFU/mL [12], meaning that our device alone will not be able to detect concentration values on the level of safety standards. However, combined with other currently available methods for raising the *E. coli* concentration in a fluid sample, such as culturing or using hydrodynamic methods to filter out the agglutinates

prior to using the detection method, our device can be used to detect *E. coli* concentrations in these ranges.

Some factors considered when designing the detection method were affordability, robustness, ease of use, and accuracy of determining the concentration of *E. coli* within the fluid sample. The detection method must be easy to use as the target demographic for this product is people in developing countries. If the method is not easy to interpret, failure to utilize the detection method correctly could cause misreadings of the concentration and lead to people using the source of the sample fluid even though it is unsafe. In addition to the ease of use, the detection method must be robust as it may be transported through rough conditions to arrive at the site of the sample fluid. If it is breakable, it will not be suitable for use in developing countries. Affordability is also a primary concern, because developing communities often lack financial resources. Since the detection method will be reusable, it is fine to have it cost a bit more than the microfluidic chip due to its lowering price per test as more tests are completed.

4.2 Options and Trades

In determining the design qualities necessary for the detection method, detection time, ease of use, size, accuracy, human interaction, fabrication accuracy, and portability were taken into account. With these criteria in mind, three detection ideas were evaluated: optical detection, electrical impedance detection, and concentration chamber detection. The optical detection method was pursued first, as it seemed to be the most promising of our choices. The other methods were thought of as backup methods and could be pursued in the case that the optical detection method turned out to be more demanding than expected. However, from a preliminary analysis, the tradeoffs of all three methods were able to be determined.

In the optical detection method, a light intensity mapping of a video of the fluid sample flowing through the microfluidic chip is obtained with the user's smartphone and the concentration of the fluid is determined through a MATLAB script that analyzes the video. The main advantage that the optical detection method has over the other methods is it provides an image of the fluid sample without the need for any external equipment. Also, the only piece of technology used in

this method is a smartphone, which are becoming more widespread in developing countries. In choosing a method other than the optical method, the capability of having an image of the sample and having a convenient power source is lost.

To utilize the electrical impedance detection method, an external electronic apparatus is required to measure the impedance of the fluid sample and determine the *E. coli* concentration from the measurement using a calibrated scale. Since this method was not attempted due to the success of the optical method, the functionality of this method is unknown; however, it is possible to alter the electrical properties of the latex particles, leading us to believe this is a valid method. The main advantage of the electrical impedance method is it is thought to provide more accurate concentration readings due to the possibility of amplifying electrical signals through the latex particles themselves. The latex particles can provide a resistance or capacitance that is readable and more sensitive than just checking impedance values through the *E. coli* particles themselves. The main disadvantage in using this method is the need for modifying latex particles to amplify the signal and the need for an external power source, as a smartphone may not have enough power to supply an electrically based detection method. In this way, the ease of use of the product may be compromised due to the introduction of electrical components and concepts that users may not understand.

In pursuing the chamber analysis method, the concentration of the fluid sample can be determined by analysis of the reactions between the latex particles and the sample fluid seen in multiple mixing chambers with different amounts of latex particles. In analyzing the chambers, the user will look for the chamber that seems to have become saturated due to the ratio of *E. coli* cells to latex particles. The main advantage of the chamber detection method is it does not require external apparatuses to determine a concentration, only a microfluidic chip. However, the interpretation of the results may be subject to the user's discretion, as it is up to the user to determine which chamber seems to be saturated. The chamber detection method also requires more detection particles for each individual test, increasing the cost of the detection method per test. In seeing these disadvantages, the chamber detection method was thought to be less accurate and more expensive over time than the other methods.

Overall, by determining the tradeoffs of each detection method, a clearer understanding of which detection method would best suit developing countries' needs was provided. The favored detection method from this analysis was the optical detection method, and therefore, the optical detection method was the first to be pursued. To justify our selection process, see Appendix B for the concept scoring matrix.

4.3 Design Description

After deciding to pursue the optical detection method, the first step was to understand the necessary parts and the design. Initially, it was recognized that the detection process would need three main parts other than the microfluidic chip: a lens to magnify the optical signal from the agglutinates, a camera to collect the optical signal from the agglutinates, and an algorithm to calculate the sample fluid's *E. coli* concentration. In combining these three components, an affective optical detection method could be implemented into our product.

To begin, it was crucial to find a proper magnification at which to capture the analysis video from. This is because the results from the video analysis will depend on the magnification used when analyzing the video. If the magnification is too low, the agglutinates will appear small in the video. This will cause inaccurate results due to pixelation—meaning a single pixel will represent a large portion of the video area. Because of this, the signal from the agglutinates in the video will be drowned out by the rest of the features in the pixel area, causing low to no signal from the agglutinates in the video. However, if the magnification is too high, the agglutinates will appear too large in the video, causing the signal from the video to be inconsistent. This is because the signal from an agglutinate will be amplified to a measure that will be uninterpretable. Therefore, the magnification lens incorporated into the detection method should have a magnification that allows for consistent results from similar detection tests. Other requirements for the magnification lens are portability and affordability. In designing or choosing a lens, it is important for the lens to be small enough to enable users to benefit from the product without needing an entire laboratory. Likewise, the lens should be cheap enough for users in developing countries to afford the product. In order to retain both of these qualities, a small attachable lens

for smartphones or a polydimethylsiloxane (PDMS) lens could be used. The advantage of utilizing PDMS material in the design is that it costs $0.01 per lens, greatly reducing our costs.

The second important component of the optical detection method was the camera, which was necessary for collecting the optical signal from the agglutinates flowing through the channel. In considering the camera, there was only one realistic option if the detection method was to remain portable and user-friendly: a smartphone camera. As previously stated, smartphones are becoming more common in developing countries, making them an incredible tool to incorporate into our detection method. Aside from their familiarity, portability, and robustness, smartphones have the capability of utilizing computer power through the use of smartphone applications, which may be created for specific purposes. This ability was capitalized on in the last important component of our detection method, the algorithm.

In designing the algorithm for analyzing the signal from the agglutinates and calculating the *E. coli* concentration, it was important that the program was quick, accurate, and could be compiled into a smartphone application. To actually gain a concentration reading from the video of the flow in the microfluidic chip, an analysis was to be completed on the light intensity from the pixels in the video. This ultimately would allow the algorithm to recognize changes in the video and acquire similar optical data to what a human would gain from looking at the sample; however, the algorithm would be much more precise in its measurements, as it would be able to see changes that could not be seen with the naked eye. Because of this, error due to human subjectivity of the results was able to be eliminated from the detection method. Therefore, in using an algorithm compiled into an application, a quick, accurate, and portable detection method was able to be created with little user interaction necessary to analyze the flow video.

In creating a design description, the three main components of the detection method were identified. This provided the team with a better understanding of what needed to be accomplished to develop a purposeful *E. coli* detection method for developing countries.

4.4 Drawings

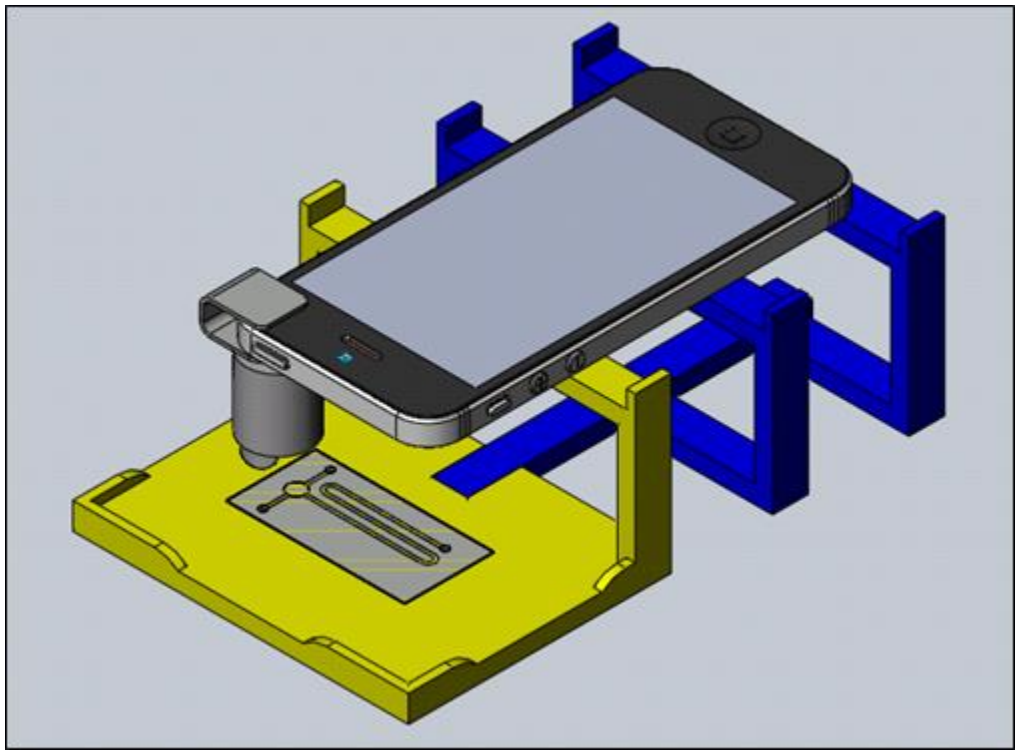

Figure 4.1: A sample configuration for the chosen optical detection method.

A sample configuration of the optical detection method in mind may be seen in Figure 4.1. As shown, the main physical parts of the detection system are the smartphone, microlens, and microfluidic chip. The stand shown in the figure is not necessarily required in the system; however, some sort of support to hold the phone while it makes its analysis would be incredibly useful in the optical detection method. In addition to providing support to the phone, it also allows for a consistent focal distance for the magnification lens to focus. Therefore, this setup served as a reminder of the end goal for the detection system.

4.5 Materials and Methods

As far as the materials for the detection method, there were only two components that needed to be obtained: a microlens and a working algorithm. In obtaining the microlens for the method, the only real plausible solution was to purchase a small lens or objective designed for using on a

smartphone. Fortunately, these lenses were low cost and readily available with different magnifications at various online vendors. The other idea that was attempted for gaining a lens was to fabricate a lens using purchased optical parts or PDMS. Since none of the members in our team were savvy with optics, building an optical piece that was suitable for our application was rather difficult. Also, attempting to create a lens made from PDMS produced two main problems. In the first, the magnification of the lenses was found to be inconsistent between the lenses. In the second, bubbles were found to form in lenses during fabrication, causing the image on the smartphone to appear blurry. This ultimately lowered the quality of the analysis video and added error to the results from the detection method. In seeing these dilemmas, it was agreed that the microlens should be purchased from an online vendor, in order to assure the lens provided a clear image and a desired magnification.

To begin creating the algorithm for the detection method, MATLAB was chosen as a platform, as it was the most familiar program to the members of our group and was able to handle image and video processing. It was also decided that the algorithm should be able to make a concentration calculation of the sample fluid using intensity data from the pixels found in a video taken with the user's smartphone. Initially, the algorithm was planned to be as simple as possible to allow for a quick calculation time; however, in actually writing the code, it was found that the algorithm would produce more accurate results with a few additional steps.

After taking the video, the algorithm assigns one of the frames—typically the first frame of the video, where there is no fluid flowing through the channel in the video—as the "baseline frame". An example of a baseline frame can be seen in Figure 4.2.

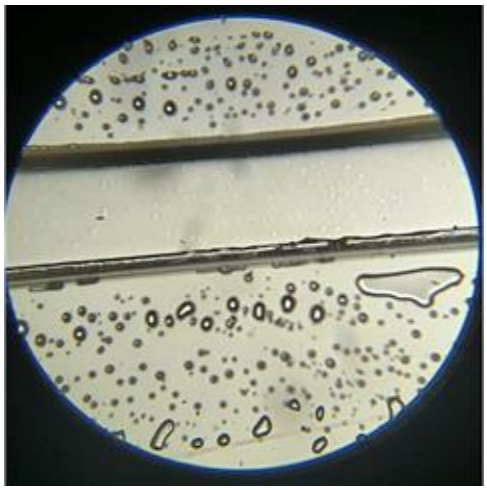

Figure 4.2: An example of a baseline frame for the algorithm. It is crucial for the baseline frame to be taken prior to the flow appearing in the 0.2 mm wide channel.

This frame provides a reference point for what the algorithm will be analyzing and allows the algorithm to see changes in the frames throughout the video, making it crucial that the flow has not yet made it through this part of the channel. After a baseline frame has been chosen, the entire video is grayscaled, combining the pixel data relating to the colors in the video into different shades of gray, which simplified the analysis. The algorithm then finds the channel inside the frames of the video by looking for which pixels in the video change the most. Using statistical analysis, the algorithm is able to find which pixels change the most between the frames, and since the camera and microfluidic chip are assumed to be still throughout the video, the only change in the video should be due to the fluid or agglutinates flowing through the chip. This ultimately allows the algorithm to find the channel in the video, as demonstrated in Figure 4.3.

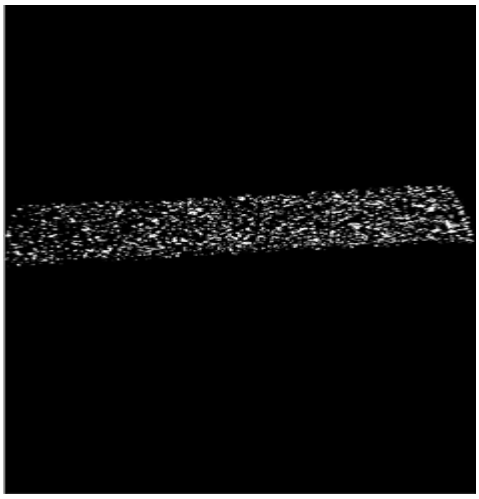

Figure 4.3: An example output of the algorithm demonstrating its ability to find the 0.2 mm wide microchannel in the video. The white pixels indicate the location of the channel in the video.

After finding the channel within the video, a small box is created inside of the channel using more statistical analyses. This box indicated where the measurements of the pixel intensity would take place. In doing this, much of the noise found in the other parts of the video, which were not relevant to the analysis, were eliminated from the results, causing test results to be more accurate and consistent. Figure 4.4 demonstrates the algorithm's ability to successfully draw a box within the channel in a video.

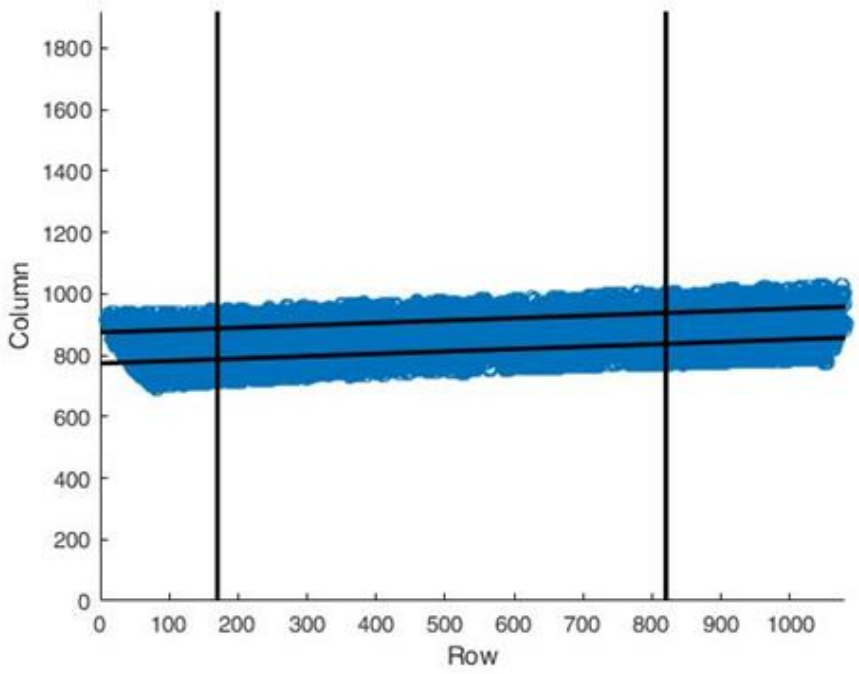

Figure 4.4: A demonstration of the algorithm's ability to draw a box inside the 0.2mm wide channel for collecting data. The shaded portion in the figure represents the changing pixels in the channel.

It should be noted that in the process of creating the cropping box, the algorithm would sometimes flip the orientation of the video, depending on if the user filmed the video with the channel facing vertically or horizontally. This was done to allow for consistent measurements and to further simplify the code. This also explains why the columns and rows are swapped in Figure 4.4, as the labels are referring to the original orientation of the video. Once the box has been created, the intensities of the pixels inside of the box from are added up for each frame and the intensity of the pixels inside of the box from the baseline frame is subtracted from each frame's intensity measurement. Since the microfluidic chip is transparent, the background surface that the microfluidic chip lies on may affect the intensity of the video. Therefore, in subtracting away the baseline frame, these effects are eliminated from the analysis, causing less error in the results. After subtracting the baseline frame intensity from each frame's intensity, the average pixel intensity of each frame is calculated by dividing the resultant frame intensity by the number of pixels inside the calculation box. This is the signal that is used to determine the *E. coli* concentration of the sample fluid. Since the average pixel intensity differs slightly for each

frame, due to the movement of the agglutinates, the average of the frame average pixel intensity is used to characterize a video. In the end, this value can be correlated to a specific concentration of *E. coli* using a general curve, found through experimental testing of the algorithm with fluid samples of known concentrations.

After purchasing a suitable lens and creating an algorithm to perform the stated process, results to validate the functionality of the detection method were able to be obtained. Also in gaining results, the general correlation curve between the algorithm output and the concentration of the fluid sample was could be created.

4.6 Expected Results

In proceeding with the devised detection method, it was expected that we would be able to create a general curve for determining the *E. coli* concentration in a fluid sample using an appropriate lens and functioning algorithm. The lens, which was bought from online vendors, was expected to be portable, affordable, and reliable. Furthermore, the smartphone camera was expected to have a high enough quality for the detection algorithm to gain accurate and consistent results.

In testing the algorithm, it was expected that not only would functionality be achieved, but the lower detection limit of *E. coli* of the algorithm would be low enough that the user could avoid catching *E. coli* in using the device to check their drinking water. As for a quantitative value for the lower detection limit, an approximation was unable to be made due to the uncertainty about how the algorithm would work. However, because the detection method relies on the agglutination of the latex particles and *E. coli* cells, it was believed that the detection limit would be near the concentration at which the agglutinates stopped forming due to a low quantity of *E. coli* in the sample fluid. This concentration was predicted to be about 10^5 CFU/mL [12]. In making these predictions, the actual testing of the detection method could be carried out with a result already in mind.

4.7 Backup Plan

In the case that the detection method did not function as planned, alternative methods to detecting *E. coli* in a fluid sample were recognized. As described before, the electrical impedance method and the chamber analysis method were still possible detection methods for our product. In the case that the optical detection method provided less than satisfactory results, one of these methods could be pursued.

4.8 Prototyping Results

In testing the prototype of our detection method, a few details should be acknowledged. To begin, our algorithm was the main component being tested, as the lens was purchased and the smartphone of the user was assumed to be functional, just like the one being used for our testing. Also, since the appropriate magnification of the lens to be used in the product was unknown, as the magnification was to be determined subjectively, a low cost microscope with an attachment that allowed a smartphone's camera to see down its eyepiece was used. In doing so, the channel's appearance at multiple magnifications was able to be seen and a magnification that would work best for the detection system was able to be chosen. The magnification found to work the best for the detection system was 100X. The testing apparatus can be seen in Figure 4.5.

Figure 4.5: The testing apparatus for the implemented detection method.

Regarding the algorithm, the previously described process seemed to provide consistent and distinguishable results among the various concentrations that were tested. In running multiple trials, many coding errors were found and were able to be fixed. Once the code was deemed fit for analyzing videos with a small amount of error, an attempt was made to transfer the program from computer, where it was originally written, to a smartphone application. For a variety of reasons including the lack of Android development experience and the weaker processing power of the smartphone, the created application's runtime was much longer than the computer script's. After completing some research on how to fix the problem, it seems that a better platform for the algorithm and a more polished application could efficiently analyze the videos, however the coding experience required for creating a more efficient application is out of our area of expertise. Therefore, the rest of the prototype testing was completed using the algorithm on the computer for simplicity in testing.

After testing and recording data from multiple detection trials of different concentrations of *E. coli*, a correlation curve between the output of the algorithm and the concentration of the fluid sample was created.

Figure **4.6** shows the curve that was created experimentally for correlating the algorithm's output to the concentration of *E. coli* in the fluid sample.

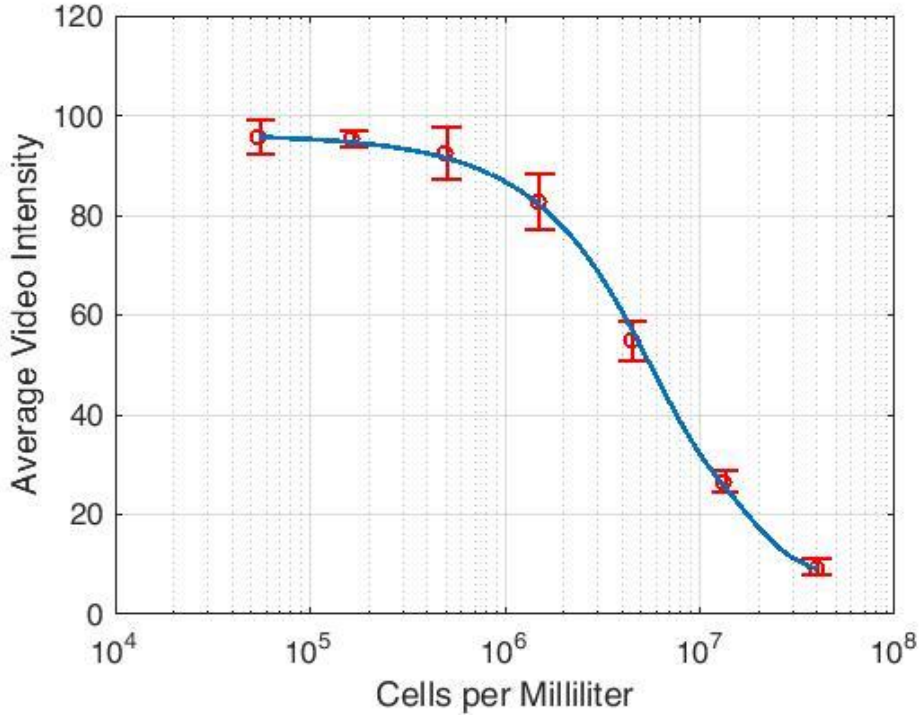

Figure 4.6: The general correlation curve between the average video intensity and the concentration of *E. coli* that was created experimentally. (Number of experiments for each concentration, n = 6)

As shown, a noticeable correlation was found between the algorithm output and the concentration of the *E. coli* in a fluid sample. It can be seen that the data points have some variance. This is believed to be caused by the change in the ambient light when the trials were completed. In order to eliminate this error, a box may be built around the apparatus when the tests are running in order to block out the ambient light in the environment. Nonetheless, the optical detection method was deemed an appropriate method for detecting and quantifying the concentration of *E. coli* in a fluid sample because a general correlation curve was able to be obtained.

4.9 Results Analysis

After analyzing the results from testing the detection method, a few conclusions were able to be drawn. To begin, optically analyzing the fluid sample for agglutinates was found to be a valid detection method that could calculate the concentration of the *E. coli* in a fluid sample. This is demonstrated by the general correlation curve that was produced using the written algorithm. Unfortunately, creating a smartphone application from the algorithm was postponed until a better coding platform was obtained; however, this was thought to be an easy fix with future work. In producing this curve, it was found that a lens of 100X magnification would provide the best results for the detection method, as the channel did not appear to small or too large in the analysis video. Therefore, a 100X microlens was purchased from an online vendor and incorporated into the system for the finished product.

The lower concentration detection limit of the optical system was also able to be estimated from the correlation curve. As expected, the curve began to level off and provide a less distinguishable output at around a concentration of 10^5 cells/mL. In seeing this, it was concluded that the detection limit for the optical system was approximately 4×10^5 cells/mL. As previously stated, the obtained limit is not low enough to detect concentration values on the level of safety standards. However, certain methods can be designed to raise the *E. coli* concentration of a fluid sample prior to its testing, such as culturing, or using hydrodynamic sorting mechanisms to filter out the agglutinates for detection. Nevertheless, the current approach provides a quantification of the traditional latex agglutination assay, which relies on visual observations and judgement of the presence of the pathogen.

Chapter 5: System Integration

5.1 Experimental Protocol

To evaluate the final product of our senior design project, the integrated system, consisting of the microfluidic chip and the detection method, must be tested. To do this, detection trials were attempted using the 3D printed stand and microlens as opposed to the low cost microscope. With the completed test trial data, a general correlation curve could be created, much like the one created during the testing of the detection method, in order to validate the system. Since the detection algorithm was found to run very slowly as a smartphone application, a video may be taken with the smartphone on the stand and then analyzed with the algorithm on a computer. In doing this, more test trials of the integrated system can be carried out in a shorter amount of time.

The test trials were completed using a similar protocol to that which was used when testing the detection method. To begin, a fluid sample of a known concentration is mixed with latex particles in the mixing chamber of the chip and allowed to agglutinate for 8 minutes. The chip and the smartphone—with the microlens attachment—are then placed onto the stand and prepared for the detection process. After, the manual valve is released and the smartphone begins to record the analysis video. Once a minute long video has been recorded of the fluid passing through the channel, the video is uploaded to the computer and analyzed with the detection algorithm. The data from multiple test trials of fluid samples with different *E. coli* concentrations is then used to create the correlation curve for when the detection method is used with the 3D printed stand. After this is completed, an analysis on the lower detection limit of the product can be completed. In completing this experimental procedure, safety is the top priority. Therefore, gloves and safety goggles were used in the experimental process.

5.2 Predictions

Prior to testing the finished product, some predictions were made about the performance of the integrated system. Since the detection method had only been tested with videos taken from the

microscope, which had no background textures to be seen through the transparent microfluidic chip, it was predicted that the results obtained from the integrated system's detection trials would have slightly more error than usual due to the background texture. However, since the stand used in the final design held the microfluidic chip on a solid, opaque platform, the background texture would be consistent throughout all trial tests. Despite the background noise, it was predicted that the detection method would function properly on the stand, as the fundamental components of the system functioned properly by themselves.

Because an enclosure for the stand was unable to be built due to project time constraints, it was predicted that the general correlation curve generated from experimental test trials would be slightly different than the one obtained when using the microscope. This is because the ambient light in the room and the background texture of the stand would slightly alter the results. Nonetheless, it was predicted that a correlation curve with a similar lower detection limit would be able to be created.

5.3 Tests and Results

A lens of proper magnification was not able to be obtained on time due to false advertisement of technical specifications of magnification lenses and delivery delays. However, for initial testing several lenses were obtained, a 60X magnification lens was used for this test. The 60X lens did not provide a high enough magnification for a viable image. The lens was then replaced with a 200X lens and a better resolution image was produced, however it was too difficult to operate due to multiple degrees of freedom in adjusting it. Due to time constraints, work on the stand and magnification lens system was suspended. The microscope based system has been demonstrated to be able to provide a viable image and has been used in the setup and generation of a correlation curve with a lower detection limit of 10^5 cells/mL. A correlation curve using the setup with a stand and a smaller magnification lens was not able to be generated due to the setup not being able to produce a viable image for analysis. The image produced by the stand was not to the correct magnification.

5.4 Comparison to Predictions

It was predicted that a correlation curve similar to that of the microscope system would be generated using our integrated setup, however no correlation curve was generated because a suitable magnification lens was not obtained. Additional work needs to be done in trying different magnification lenses until a suitable one is found to create an image that is able to be analyzed by our detection algorithm. Future plans would be to have an upcoming senior design group continue our project to finish fully integrating our system into a stand with a magnification lens.

Chapter 6: Cost Analysis

For the purpose of prototyping, the ARFLOW 93049 hydrophilic adhesive was provided free of charge by Adhesives Research. This meant the primary material costs for the prototypes were glass slides, latex particles, and other adhesive materials that were tested.

The main bulk of the project cost was the Graphtec CE6000 cutting plotter that can be used for both prototype fabrication and finished product production. For the duration of the project, maintenance costs were not expected for the CE6000; however, for long term production, maintenance costs must be accounted for.

Lysogeny broth for culturing *E. coli* bacteria for testing the effectiveness of our device is only necessary for the duration of our project and is not necessary for long term production except for potential quality testing.

The production of the microfluidic chip itself, which consists of the hydrophilic adhesive and a glass slide, is less than $1 per test, as the glass slides are at most $0.50 each and can be reused if necessary. In comparison to the overall budget of $1700.00, each prototype is extremely cheap to make and costs less than $1.00 per test. The main cost associated with the production of the chips is the initial fee of buying a Graphtec cutting plotter. Since it is a relatively cheap machine for $1100.00, the buy-in price to produce these chips is low for any companies interested in the mass production of this product. Production costs are relatively low as the manufacturing requirement only requires the deposition of the pre-cut adhesive and latex particles onto a glass slide, along with the manufacture of the detection method.

Like the microfluidic chip, the detection method was able to be implemented into the final design for a low cost. The final detection method was a reusable optical detection method that utilized a low cost microscope, a smartphone, and latex particles. While the microscope cost $150.00, it was able to be reused for multiple tests, and therefore, the cost per test of our detection system would decrease significantly over time. However, plans were made to replace the low cost microscope with a microlens and a 3D printed stand. In doing this, the cost was estimated to drop

significantly, as microlens can be purchased for less than $15.00 and the 3D printed stand can be made with very little material cost (72.45 cm^3 of plastic). Additionally, a lens made from PDMS may be implemented with further research, causing the cost per lens to drop to about $0.01. As for the smartphone, the user is responsible for providing this causing there to be no cost from this component. Lastly, latex particles may be purchased at about $0.50 per test, allowing tests to be affordable for people in developing countries.

Therefore, the overall initial prototype cost, which includes the reusable detection method and a microfluidic test chip, was estimated to be around $151.00. However, as stated before, multiple tests may be carried out for this price, causing the price per test to be approximately $1.00 for the benefit of developing countries. Furthermore, this cost can be greatly reduced in replacing the low cost microscope for a microlens or PDMS lens.

Chapter 7: Business Plan

7.1 Introduction

Waterborne disease caused by contaminated water remains an important global health issue, particularly in developing nations. Testing water samples is conventionally performed in laboratories off-site with expensive equipment and takes days or weeks to return the results. This makes the technologies used not applicable for testing water supplies in rural regions. However, in recent years, there has been a push towards point-of-care testing (POCT), which allows for a quick, on-site diagnosis in a variety of biomedical areas, particularly within developing nations. In seeing this push, The World Health Organization (WHO) has established seven guidelines for the development of diagnostics in resource-limited settings. They say the diagnostic tests for the developing countries should be "ASSURED" – affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to those in need [1]. With these guidelines, we propose to develop a low-cost, autonomous, miniaturized fluidic device capable of detecting pathogens in fluid samples, which will be useful for on-site environmental monitoring, especially in developing nations. Our device will be able to drive a flow with capillary force, eliminating the need for an external pumping system. The detection method used with the microfluidic chip will also be designed in such a way that no additional equipment will be needed for the detection of the pathogens to take place. This ultimately will limit the overall cost and complexity of the system and allow the device to comply with the "ASSURED" guidelines of WHO.

The discoveries enabled by our device can also translate to diagnosis. WHO also notes syphilis and hepatitis A and B to be major problems in Africa [2]. These diseases could be tackled using our device with slight modification. If a patient walks into a hospital with severe gastroenteritis, the cause could be one of many. A simple test with this device could potentially lead to quick detection of the pathogen at fault. This will greatly reduce the cost of medical care and results should come within a matter of minutes.

The fluidic platform developed in this research provides a low-cost, user-friendly tool to detect pathogens in fluid samples, which can directly benefit rural regions where there are no programs for testing water supplies. Allowing such regions to test their water before using it will enable the less fortunate to avoid illness through the use of contaminated water, improving the health of these users. This ability—to sustain good health—is not only for the less fortunate, but it is a benefit to humanity in general, as our product can be used to test water in developed regions as well.

If our device were to be commercialized in a manner to reach everyday people, the overall safety of the public could improve.

7.2 Goals and Objectives

We intend our company to be a non-profit organization with the goal of improving the living conditions in developing rural communities by allowing these communities to test their water sources for *E. coli* contamination in an affordable and simple way. Allowing rural developing communities to test their water for *E. coli* contamination will reduce the prevalence of *E. coli*-related diseases by allowing community members to safeguard themselves from the contaminated water and will allow government and humanitarian organizations to deliver aid to affected communities. It is also our goal to develop our detection device further such that it can detect pathogens other than *E. coli* and therefore further improve the quality of life in communities affected by other types of water pathogen contamination.

7.3 Description of Product

Our product is a two-component system designed to measure the concentration of *E. coli* bacteria in water. The two components of our device are a microfluidic chip platform and a detection method.

The microfluidic chip platform is intended to control the flow of fluids necessary to detect *E. coli* contamination as well as provide a platform for which the detection method can be used. It consists of the following layered build:

- A bottom layer of transparent glass
- A middle layer of transparent hydrophilic adhesive tape with channels cut into it
- A top layer of transparent hydrophilic adhesive tape with inlet and outlet holes cut into it

To amplify the *E. coli* signal, latex particles that can bind to *E. coli* to form agglutinates are mixed with the water sample. The microfluidic chip platform allows for the necessary fluids to be input in the fluid inlets, which allows the fluids to be mixed. A scotch tape valve prevents fluid from leaving the mixing chamber until the user opens the valve. Once the valve is opened, the fluid flows through a detection channel where the detection system can measure the *E. coli* concentration with the use of a smartphone and magnifying lens.

The detection method for this system consists of a computer algorithm that analyzes a video from a smartphone and magnifying lens of the working fluid flowing through the detection channel of the microfluidic chip. This algorithm optically measures the light intensity of the video. For different *E. coli* concentrations, there will be different intensity values. The algorithm then correlates the measured intensity to *E. coli* concentration and outputs a range of *E. coli* values.

Our detection method can be downloaded as an app on a smartphone and our microfluidic chips can be sold separately.

7.4 Potential Markets

Pathogenic *E. coli* is a global problem that affects both the developing and developed world. In the developed world, there already exist technologies and methods that are useful in detecting *E. coli* contamination in water; however, these technologies and methods are not practical in many developing rural communities. The methods used for the developed world are too expensive,

require too much training, and require too much infrastructure to be a practical means of detecting *E. coli* in the developing world.

Our device is more practical for use in the developing world, because it is cheaper, simpler, and requires less training and equipment than many other detection methods that currently exist. Another significant advantage that our device has over other affordable methods and devices that currently exist is that our device can quantify the contamination of *E. coli* in water, as opposed to other devices and methods that can only output a rather subjective binary reading.

The primary markets that we seek to establish ourselves in are developing rural communities in Latin America, Africa, and Asia. Within these regions, we aim to focus more heavily on communities and nations that have histories of unsanitary water, because these are regions that are more likely than other regions to be affected by *E. coli* contamination. In these communities and nations, potential customers would be governments, health organizations, and humanitarian organizations. Governments can include national governments, provincial governments, city governments, and village governments.

In addition to organizations based within the regions mentioned previously, we also seek to target organizations based outside those regions, but that still work within the regions in question. One such organization would be the United Nations World Health Organization, which is based in Switzerland. They are a potential customer, because much of their work is done in the developing world where the infrastructure and personnel necessary to operate traditional detection systems are not readily present.

In order to fit the needs of customer organizations of differing size, we will sell our microfluidic chips in packs of 10 and 100. Smaller organizations may prefer to purchase in increments of 10 as opposed to large organizations that might seek to buy on a much larger scale in increments of 100.

7.5 Competition

Three major competing products have been identified. They are the following:

- **Filters Fast Detection Kit [8]:** This is a method by Filters Fast that allows the user to mix a water sample with a reactant fluid. If there is a contaminant in the water, the solution will change color. This system takes 48 hours to deliver results at $10 per test. No additional equipment is required to perform tests meaning the system is very portable. This method does not quantify the pathogen contamination and cannot specifically target *E. coli*. Additionally, this system has a relatively high lower detection limit, meaning it cannot detect small levels of contamination. Ultimately, this device is reasonably well-suited for developing rural communities due to its simplicity and ease-of-use; however, its detection time, cost, inability to specifically target *E. coli,* and inability to quantify contamination make prevent it from being an ideal method of detecting *E. coli* in the developing world.
- **Salmonella Detection Research Product [9]:** This is a method in development that detects *E. coli* at a very high level of accuracy with a very low lower detection limit in 10 minutes for less than $1 per test; however, this method requires a lot of equipment, including optical systems and lasers in order to perform its analysis. Additionally, this device can quantify the level of *E. coli* contamination. Despite its high level of precision and accuracy, this device is not well suited for developing rural communities due to the need for expensive laboratory equipment and trained personnel.
- **Traditional Agglutination Test [13]:** This is a method that allows a user to mix a water sample with latex particles. If at least 10^5 cells/mL of *E. coli* are present, the latex particles will agglutinate, forming clumps visible to the naked eye. This method takes approximately 3 minutes and costs less than $1 per test. No substantial equipment is required for this method and the system can specifically target *E. coli*, but it does not have the ability to quantify the contamination. The traditional agglutination test is well-suited to rural developing communities due to its simplicity, ease-of-use, and affordability, but its inability to quantify the contamination makes it less ideal than our own product. We consider this product to be our primary competitor in our markets.

Table 7.1: Summary of comparison of our product and other *E. coli* detection products.

	Filters Fast Detection Kit [8]	**Salmonella Detection Research** [9]	**Traditional Agglutination Test** [13]	**Our Product**
Detection time	48 hrs	10 mins	3 mins	<10 mins
Cost per test	$10.00	<$1.00	<$1.00	~$1.00
Cost for equipment	$0.00	High (Optical system)	$0.00	$150 (Microscope)
Portable	Yes	No	Yes	Somewhat
Quantifiable	No	Yes	No	Yes
Specific	No	Yes	Yes	Yes
Lower Detection Limit	High	Low (10^2 cells/mL)	High (>10^5 cells/mL)	High (10^5 cells/mL)

7.6 Marketing Strategies

To market our product, having it endorsed by multiple health and humanitarian organizations responsible for areas of our target market would greatly improve people's perceptions of seeing our product as practical. For example, endorsements by organizations such as the African

Medical and Research Foundation (AMREF), would give our product more credibility in the eyes of people in the developing rural communities of Africa. If other large health organizations were to do the same, our product would be exposed to the eyes of many.

Salespeople associated with our product be situated in areas in which our product is needed as they would be able to demonstrate how the quickly the product works in person. Salespeople would also persuade customers that the worry of not having a drinkable water source due to *E. coli* contamination would disappear with the use of our product. As we aim to sell a complete system with disposable tests which the customer would purchase more when needed, there would only need to be 5-10 salespeople in order to sell the system in developing communities and purchase of more tests can be done through online sales.

7.7 Manufacturing Plans

The only component of our system that requires manufacturing is our microfluidic chip. Our chips will be manufactured in a developing country in order to minimize manufacturing costs while also contributing to the development of the country in question. Required equipment for manufacturing includes a plotting cutter, forceps, and padded rollers.

Microfluidic chip channels will be cut into hydrophilic adhesive tape with a plotting cutter and then workers will remove the hydrophilic adhesive tape layers and attach them to glass slides. Tweezers can be used to assist in peeling hydrophilic adhesive tape layers from their plastic base. Padded rollers can be rolled on the chips to ensure that the hydrophilic adhesive tape layers are securely attached to the chip. These tasks will be separately assigned to workers to develop a production line.

Each chip is capable of performing 3 detection tests and will take an average of 45 seconds per worker to assemble, meaning that 80 chips can be assembled in an hour per worker. Assuming a manufacturing operation of 3 workers, approximately 1,800 chips can be manufactured in an 8 hour work day. Ramp up time for our operation would be very short (approximately 3 minutes) and is therefore negligible.

Each chip is 2in x 3in x 0.04in, or $0.24in^3$, meaning that a 4ft x 3ft x 6ft storage area can store 20,700 chips, excluding the space required for boxes or dividers. This is as much storage as would be needed, because this is as much storage as would be needed to store 2 weeks worth of production.

The initial amount of money that would be required to buy the required equipment, supplies for a full inventory of chips, and pay workers assuming a $3 minimum wage would be approximately $15,500. This does not include costs associated with obtaining a space for manufacturing.

In order to expand and continue to be able to sell chips, we must develop our detection method for use on other pathogens, such that our chips can be used for additional functions.

7.8 Product Cost and Price

The cost of our microfluidic chips can be divided into fixed, per-unit, and monthly costs. These costs are the following:

- Fixed Costs
    - Plotting Cutter: $1000
    - Forceps and Padded Rollers: $50
- Per-Unit Costs
    - Glass Slides: $0.50
    - Hydrophilic Adhesive Tape: $0.25
- Monthly Costs
    - Space Rental: $300 (Based on rental costs in India with utilities included)
    - Worker Wages: $504 (Based on 3 workers working 40 hour weeks at $3/hr)

Based on the costs above and an assumption that 3 workers can produce 40,000 chips in a month, the total costs per chip are cited in Table 2.

Table 7.2: The costs per unit for each chip in relation to time span.

Time Span (months)	1	3	6	9	12	24
Cost per Unit	$0.80	$0.78	$0.77	$0.77	$0.77	$0.77

Based on these values, our product is very competitive in price to the traditional agglutination test, which costs approximately $1 per unit. This is very good, considering our product is an improvement on the traditional agglutination test.

7.9 Services or Warranties

As we predict our product will be robust and cheap to produce, we would like to guarantee that each chip in our 10-pack and 100-pack of chips functions properly. If a pack contains defective chips, the customer can notify our company and we will send them replacements for the defective chips. Replacements will come in increments of at minimum 10, because that is the smallest increment at which we sell our chips.

7.10 Financial Plan

In order to begin our operation, we will require $60,000 to purchase supplies, hire workers, contact customers, and rent a manufacturing space. This money will be raised through grants from organizations such as the United Nations World Health Organization. Additionally, funds can be borrowed from banks.

Assuming that 40,000 chips are manufactured in the first month of operation and that each chip is sold and sells at an average $1.20, then the initial investment will be paid off in less than two

months. The assumption that all the chips will be sold is dependent on our ability to make connections with customers before production begins and that these customers will purchase our inventory.

Our company is expected to have a quick return on investment (2 months), after which the profit gained from selling chips can be used towards developing our detection method and marketing.

In the event that sales are less than expected for our microfluidic chips, a possible contingency plan would be to identify potential customers in academia that would be interested in having their own hydrophilic adhesive tape microfluidic chips for research purposes. We could then reduce production of our regular chip designs and produce the designs desired by customers in academia. This contingency plan is plausible, because building alternate designs is very simple and does not require any sort of retooling.

Chapter 8: Engineering Standards and Realistic Constraints

8.1 Economic

Many developing countries have a high prevalence of poverty that makes it difficult for communities to afford the necessary materials, equipment, and personnel to perform accurate and precise water contaminant testing. These communities also often lack the infrastructure necessary to perform these tests using traditional laboratory equipment.

Designing a device that is both usable and affordable for such communities is of great importance for our project. Our design process considers methods of minimizing the cost of our device for these communities. Two important factors that affect the affordability of our device are material costs and production costs. To address these considerations, we have designed a system using low cost materials to use cheap materials such as glass or plastic slides, ARFLOW 93049 hydrophilic adhesive, and scotch tape for our device.

Considering the economic reality of our customers, we have also sought to ensure that our device is usable with the infrastructure that exists in these communities and that our device is easy enough to use such that little to no educational background is necessary to use our device.

Providing poor communities with an affordable method of detecting bacterial contamination in their water supplies has the potential to enhance their economic growth by reducing medical costs and improving the health of these communities.

8.2 Health and Safety

A number of diseases commonly found in developing countries are caused by the consumption of unsanitary water. One way to prevent infection from these diseases is to identify contaminated water sources and warn individuals of the risks from consuming water from these contaminated

sources. In order to do this, it must be possible to detect contamination in water with as much accuracy as possible. Accuracy is important because a false-negative reading has the potential to cause great harm by creating a false sense of safety regarding a contaminated source of water.

Our device not only seeks to detect contamination in water, but also seeks to do so as accurately as possible. An accurate method of detecting contamination in water can reduce the prevalence of preventable disease caused by contaminated water consumption by warning people beforehand of contamination. Being able to detect the concentration of contamination can also provide valuable data that would be useful in identifying the source of the contamination so that further action can be taken to eliminate the threat.

8.3 Sustainability

Sustainability of a product is an important issue in the development of products for developing countries, as they may not have established systems for safe disposal of materials. In order to create a sustainable product, the product should not disturb the natural ecosystem around when it is disposed of. To prevent any unnecessary waste in the environment, one consideration in designing our device is to make it biodegradable or recyclable. By making the single-use microfluidic chips recyclable or biodegradable, people using the product would feel as though they were making contributions to their communities while maintaining their responsibility towards their environment.

Additionally, we seek to build our device with materials that do not have a significant environmental impact to acquire, such that our device contributes to a future that we can sustain. The point of making a product sustainable is to encourage the use of the product without any lasting negative effects to the environment.

8.4 Social

One of the issues that exists when determining the best method to economically develop a community is the issue of dependence. This can be easily summed up in the common saying "give a man a fish and you feed him for a day. Teach a man to fish and you feed him for a lifetime." The parallel that can be drawn here is the difference between performing contamination tests for developing communities versus giving developing communities the tools necessary to perform their own tests. In order for developing communities to build a future that lasts, its people must feel like they are a part of building that future. The absence of this has the potential to create a society of people who do not truly feel in charge of their own destiny. We seek to empower the individuals living in these poor communities by giving them the ability to test their own water for drinkability.

8.5 Ethical

Our project seeks to design a device that has a net positive ethical impact on the people and communities that use it. We believe that our device is ethical in two major ways: it will directly reduce the likelihood that individuals will drink water contaminated by biological agents and it will indirectly create a more socially just world.

When it comes to social justice, one of the biggest problems is the inequality between individuals living in developed economies and individuals living in developing economies. Many technologies exist today that can ensure the safety of water for consumption; however, these technologies are either unavailable or very difficult to obtain for poorer communities around the world. Obstacles in these communities include lack of affordability, lack of infrastructure, and lack of necessary training and education. As a result, many of these communities are faced with serious health concerns in regards to contaminated water, which directly and indirectly perpetuate the poverty in these parts of the world. By addressing the issue of contaminated water, we hope to help end this cycle of poverty and improve the health of individuals.

8.6 Arts

As part of satisfying the SCU Core Arts & Humanities requirements, mechanical engineering members of this team have all contributed original drawings, sketches, and/or CAD models and drawings to this project. Below are listed a sampling of at least one such artifact, and a reference to it, for each of the team members.

Table 8.1: Samples of drawings, sketches, and/or CAD models and drawings created by mechanical engineering team members.

Team Member	**Description**	**Location**
Andres Maldonado	A drawing of some of the channels designed to test the speed of the flow through the microchannels. (Drawn by Andres Maldonado-Liu)	**Figure D.8**
Andy Ly	A drawing of a phone case that was designed to be a detection method. (Drawn by Andy Ly)	**Figure D.5**
Kyle Pietrzyk	A CAD model of a design of the microfluidic chip and the optical detection method. (Drawn by Kyle Pietrzyk)	**Figure D.1**

Chapter 9: Future Work and Conclusion

9.1 Future Work

While we have accomplished our major goal of being able to quantify latex particle agglutination, we have yet to achieve our ultimate goal: to create a point-of-care device that will detect *E. coli* in water sample. This means that we must have a portable solution that can perform its necessary steps autonomously. To accomplish this, we have begun work in several different directions that we believe will increase the usability of our device.

Smartphone Application: The current setup relies on a computer to perform all of the necessary calculations that go into our MATLAB script. This reliance can be nullified by creating a smartphone application that can efficiently perform the same analysis of the MATLAB script on an Android platform. As of current, the only progress made on the application was a simple vibration setting to a desired time and access to the camera in order to take an image for our detection algorithm.

Smaller Lens: The reliance on a microscope is a major barrier to creating a portable, standalone device. We have done some work in developing a PDMS lens, which are particularly attractive because it is quick, small, and costs about a penny to manufacture. This lens can be placed on the smartphone's camera to get up to a 100x magnification. Other potential solutions include the variety of fairly inexpensive lenses that can be found online at stores like Amazon.

Enclosure: While we have seen fairly consistent results, slight variation can be seen between tests on separate days. We believe one reason for this difference is ambient light. To improve the consistency of our device, we have created a cardboard box around the desired area to be imaged as a temporary solution. A more elegant solution would be to 3D print a box specifically towards the dimensions of the stand that encases the light.

Vibration Mixing: One of our goals for this device is for it to require minimal user interaction. Our current solution has the user mix the latex particles with the sample water

before running it through the sample. To avoid this, we are working to incorporate vibration into our smartphone application so that the particles and the water can both be introduced into the system and then the phone's native vibration can be used to ensure agglutinate formation.

9.2 Conclusion

The objective of this project was to develop an *E. coli* detection device for use in developing communities around the world. For this device to be effective in developing areas, it needed to meet the ASSURED criteria as closely as possible. In addition, our device needed to be an improvement on methods that currently exist and also meet the needs from our customer needs analysis.

Our approach was to take the traditional agglutination detection method, which already met a number of the ASSURED criteria, and improve it such that it could quantify the *E. coli* concentration of a contaminated water sample.

The current version of our device consisted of two primary components: the microfluidic chip and the detection method. The microfluidic chip component of our device was intended to facilitate mixing between latex particles and sample fluid while also providing a platform for detection. The device itself consists of a layered design that uses capillary forces to drive the fluid flow. Channel geometries and valves are cut into the middle layer, which will allow for flow control as well as fluid mixing. This mechanism provides a clear advantage over other methods because it is cheap, does not require external pumps or other special equipment, and maintains a small, flat shape that allows for efficient transport and delivery.

The detection method's function was to detect the *E. coli* concentration in a water sample. Using latex particle agglutination, this device was able to target a specific strain of *E. coli* and quantify it to the level of 4×10^5 cells/mL range. While this limit is not as low as is desired, there are several methods such as culturing and hydrodynamics that may be used to achieve a lower detection limit. Also, since we have created a new method to quantify the concentration, there is much potential for improvement in the future. Currently, out method uses a 100x magnification

microscope to take a video using a smartphone and then analyzes the video using a MATLAB script on a computer. While this setup allows for the quantification of the traditional agglutination detection method, it is more costly and less portable. Integrating a mobile 100x magnification lens and a more polished Android application will address both of these issues and will result in a standalone device that can quantify and correlate latex particle agglutination to a specific concentration of *E. coli*.

In its current state, this detection system improves on the traditional agglutination detection method by quantifying *E. coli* concentration while still being simple and relatively deliverable. With further work, our system can truly be user-friendly, affordable, and equipment free.

While we are not quite at our goal of creating a point-of-care device for pathogen detection, our product is a step in that direction. We hope that further development of this product will result in a compact device available to the developing world that can detect a variety of pathogens.

Appendix A: Bibliography

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Appendix B: Product Design Specifications

Table B.1: The initial product design specifications for the microfluidic detection platform, based on the specifications of other devices of a similar nature and based on the specifications of materials used.

Specification	Value
Accuracy of Measured Contamination Percentage	10% error
Repeatability	9 out of 10 times
Overall Size of Chip	No more than 80 cm^2
Time for Measurement to be Completed	No more than 10 minutes
Cost per Unit	No more than $1.00
Usable in Developing Countries?	Yes
Shelf-Life	At least 6 months
Necessary Amount of Sample Water	No more than 100 mL
Number of Usages per Unit	1
Flow Rate	Fast enough to not take too much time. Slow enough to gain accurate results.

Table B.2: The final product design specifications for the *E. coli* detection system and a comparison to the relevant initial product design specifications.

Specification	Target Values	Actual Values
Detection time	<10 mins	<10 mins
Cost per test	<$1.00	~$1.00
Required Equipment	Smartphone and lens	Smartphone and lens
Cost for equipment	$8 [Smartphone not included]	$150.00 (Magnification lens) [Smartphone not included]
Portable	✔	O*
Quantifiable	✔	✔
Specific	✔	✔
Lower Detection Limit	10^5 cells/mL	~10^5 cells/mL

* The current apparatus makes use of a low-cost microscope for 100X optical magnification. There are several ways to eliminate the use of the microscope such as using a small PDMS lens (refer to page 74).

Appendix C: Decision Matrices

The following matrix shows some of the options that were considered for the microfluidic chip when designing the senior design project.

Table C.1: The selection matrix used to see some of the options for the microfluidic chip.

Microfluidic Chip Selection Matrix		
Aspect	Choices	Description
Top Layer of the Chip	Scotch Tape	Regular Scotch Desk Tape. Provided an even, slow flow rate that allowed for valves to be used.
	Packaging Tape	Scotch Packaging Tape. Provided an uneven, fast flow rate and too much driving force for valves to be used.
	Glass	Use two glass slides to sandwich the plastic with channels carved into them. Provided a strong driving force.
	Adhesive Plastic	The same adhesive that the channels are cut into. Provided an even, quick flow rate, but it may be too strong.
Micro Valve Type	Automatic	Use a capillary valve to stop the water flow without any human interaction.
	Manual	Use a secondary outlet that can be used as a valve and activated by taking off the tape covering it.

The following figure shows the decision matrix used to decide which material for the top layer of the microfluidic chip should be used for the final product.

Design Project =	E. Coli Detection Chip			System=	Microfluidic Chip Top Layer Material							
	TARGET or	DESIGN IDEAS										
CRITERIA	FACTOR	1 = Baseline		Scotch Desk Tape		Scotch Packaging Tape		Glass		Adhesive Plastic		
Time – Design	40	40		70		60		50		90		
Time – Build	60	60		50		70		70		90		
Time – Test	70	70		60		70		60		70		
Time Score	7		7		8.03		8.56		7.64		11.08	
Cost – Prototype	15	$ 15.00		$ 0.20		$ 0.20		$ 0.50		$ 0.80		
Cost – Production	5	$ 5.00		$ 0.10		$ 0.10		$ 0.25		$ 0.40		
Cost Score	10		10		0.17		0.17		0.42		0.67	
Proper Driving Force	5	3	15	4	20	2	10	3	15	2	10	
Ease in Implementation	1	3	3	3	3	4	4	2	2	5	5	
Cost	7	2	14	5	35	5	35	5	35	4	28	
Allows for Automatic Valves	5	1	5	5	25	1	5	3	15	1	5	
Human Interaction	8	2	16	4	32	3	24	3	24	3	24	
Robustness	4	5	20	5	20	5	20	1	4	4	16	
0	0	3	0		0		0		0		0	
0	0	3	0		0		0		0		0	
0	0	3	0		0		0		0		0	
0	0	3	0		0		0		0		0	
0	0	3	0		0		0		0		0	
0	0	3	0		0		0		0		0	
	TOTAL		73.0		143.8		106.3		103.9		93.3	
	RANK											
	% MAX		50.8%		100.0%		73.9%		72.3%		64.8%	
	MAX	143.8										

Figure C.1: The decision matrix used to decide which material for the top layer of the automatic microfluidic chip should be used.

The following figure shows the prioritization matrix used to find the weights of the evaluated qualities of the top layer material.

Project:	E. Coli Detection Chip									
System:	Microfluidic Chip Top Layer Material									
Date:	12/4/2015									
	Criterion	**1**	**2**	**3**	**4**	**5**	**6**	**7**	SUM	FACTOR
1	Proper Driving Force		1	0.5	0.5	0	0.5		2.5	5
2	Ease in Implementation	0		0	0.5	0	0		0.5	1
3	Cost	0.5	1		0.5	0.5	1		3.5	7
4	Allows for Automatic Valves	0.5	0.5	0.5		0.5	0.5		2.5	5
5	Human Interaction	1	1	0.5	0.5		1		4	8
6	Robustness	0.5	1	0	0.5	0			2	4

Figure C.2: The prioritization matrix used to find weights of the qualities of the top layer material for the automatic chip.

The following figure shows the decision matrix used to decide which detection method to use for the final product.

Design Project =	E. Coli Detection Chip			System=	Detection Methods					
	TARGET or	DESIGN IDEAS								
CRITERIA	FACTOR	1 = Baseline		Iphone Detection		Impedance Detection		Separating Valve		Concentration Chambers	
Time – Design	50	50		80		50		30		30	
Time – Build	30	30		40		30		10		10	
Time – Test	70	70		70		60		50		60	
Time Score	7		7		9.18		6.67		3.84		4.18
Cost – Prototype	15	$ 15.00		$ 30.00		$ 15.00		$ 8.00		$ 10.00	
Cost – Production	5	$ 5.00		$ 2.00		$ 1.50		$ 1.00		$ 1.25	
Cost Score	10		10		12.00		6.50		3.67		4.58
Detection Time	6	3	18	5	30	5	30	3	18	3	18
Ease of Use	8	3	24	4	32	4	32	4	32	3	24
Size	5	3	15	5	25	4	20	5	25	5	25
Accuracy	6	3	18	3	18	2	12	2	12	3	18
Human Interaction	3	3	9	2	6	3	9	3	9	3	9
Fabrication Accuracy	4	3	12	4	16	2	8	3	12	3	12
Portability	5	3	15	4	20	3	15	5	25	5	25
0	0	3	0		0		0		0		0
0	0	3	0		0		0		0		0
0	0	3	0		0		0		0		0
0	0	3	0		0		0		0		0
0	0	3	0		0		0		0		0
	TOTAL		111.0		142.8		129.8		142.5		139.2
	RANK										
	% MAX		77.7%		100.0%		90.9%		99.8%		97.5%
	MAX	142.8									

Figure C.3: The decision matrix used to decide which detection method should be used.

The following figure shows the prioritization matrix used to find the weights of the desired qualities of the detection method.

Project:	E. Coli Detection Chip									
System:	Detection Method									
Date:	11/10/2015									
	Criterion	**1**	**2**	**3**	**4**	**5**	**6**	**7**	SUM	FACTOR
1	Detection Time		0	1	0.5	1	1	0.5	4	6
2	Ease of Use	1		1	1	1	1	0.5	5.5	8
3	Size	0	0		0.5	0	0.5	0.5	1.5	5
4	Accuracy	0.5	0	0.5		1	1	0.5	3.5	6
5	Human Interaction	0	0	1	0		1	1	3	3
6	Fabrication Accuracy	0	0	0.5	0	0		0	0.5	4
7	Portability	0.5	0.5	0.5	0.5	0	1		3	5

Figure C.4: The prioritization matrix used to weigh the desired qualities of the detection method.

Appendix D: Sketches

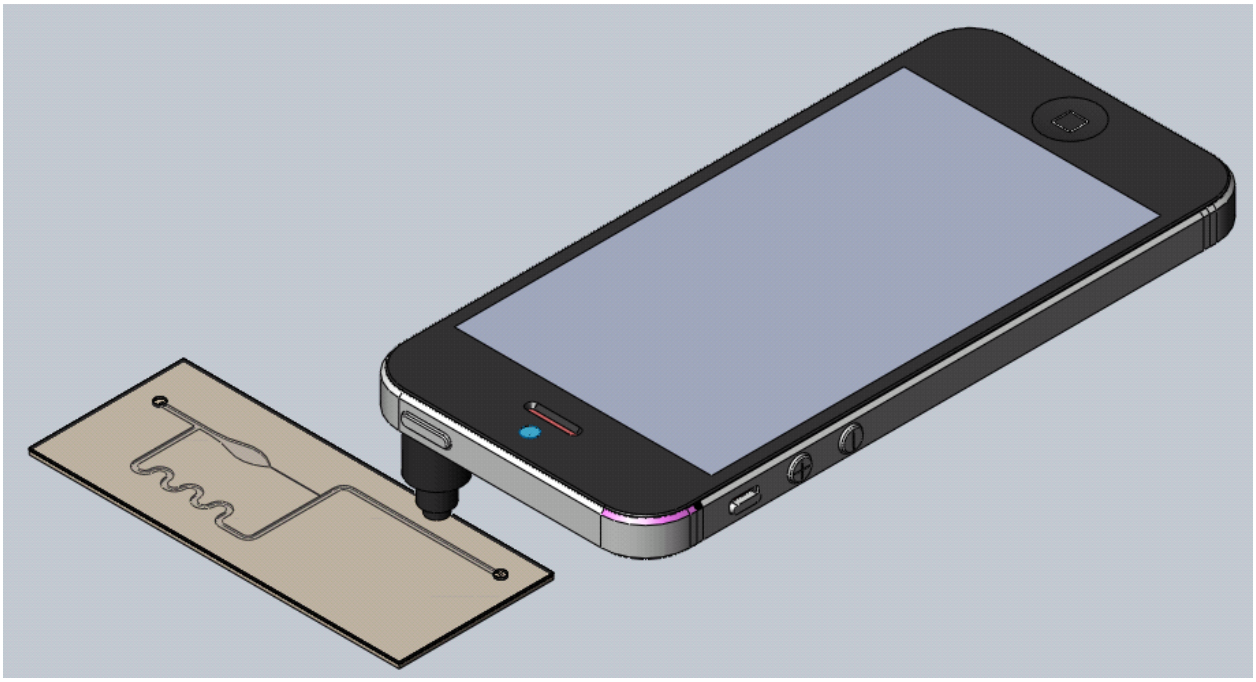

Figure D.1: A CAD model of a design of the microfluidic chip and the optical detection method. (Drawn by Kyle Pietrzyk)

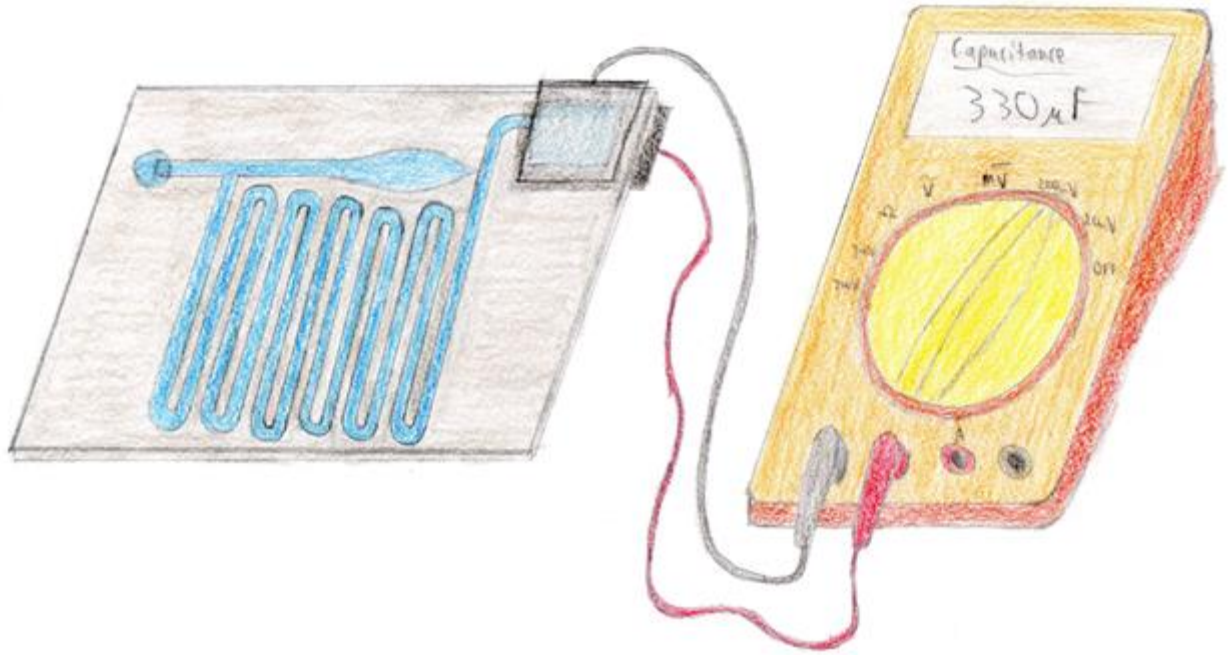

Figure D.2: A drawing of the electrical impedance detection method. (Drawn by Kyle Pietrzyk)

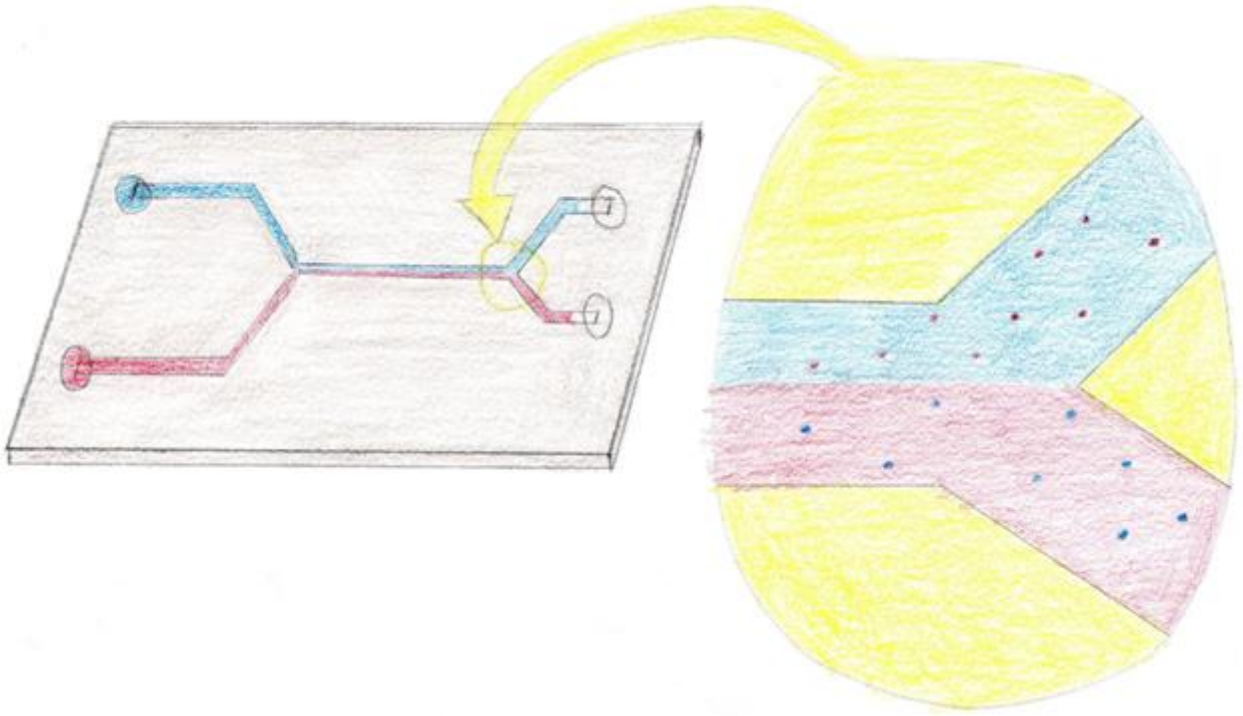

Figure D.3: A drawing of the detection method using a single channel of two fluids to pull the particles to opposite outlets. (Drawn by Kyle Pietrzyk)

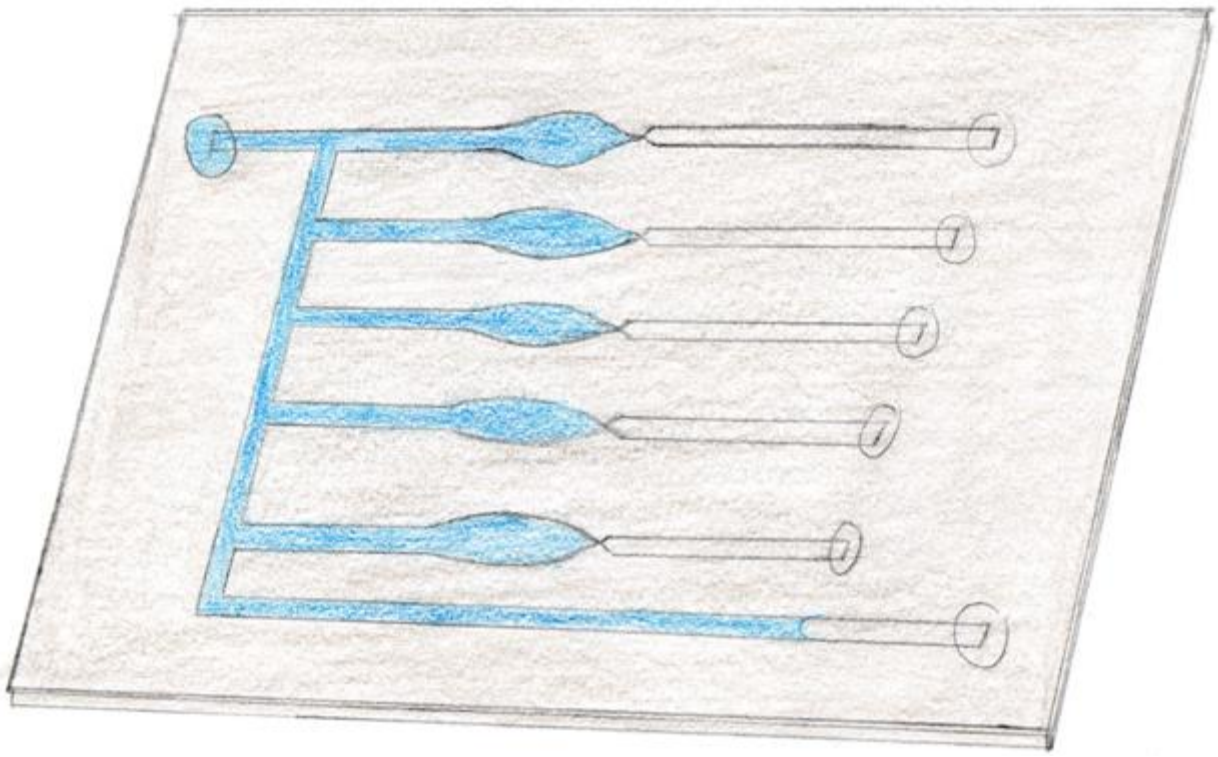

Figure D.4: A drawing of the multiple chamber detection method. (Drawn by Kyle Pietrzyk)

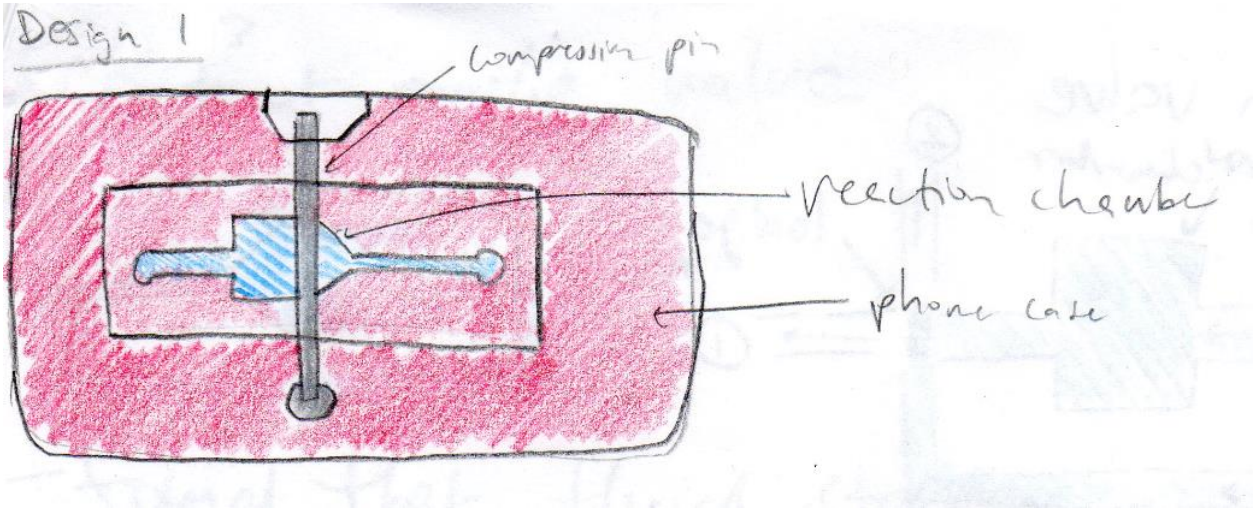

Figure D.5: A drawing of a phone case that was designed to be a detection method. (Drawn by Andy Ly)

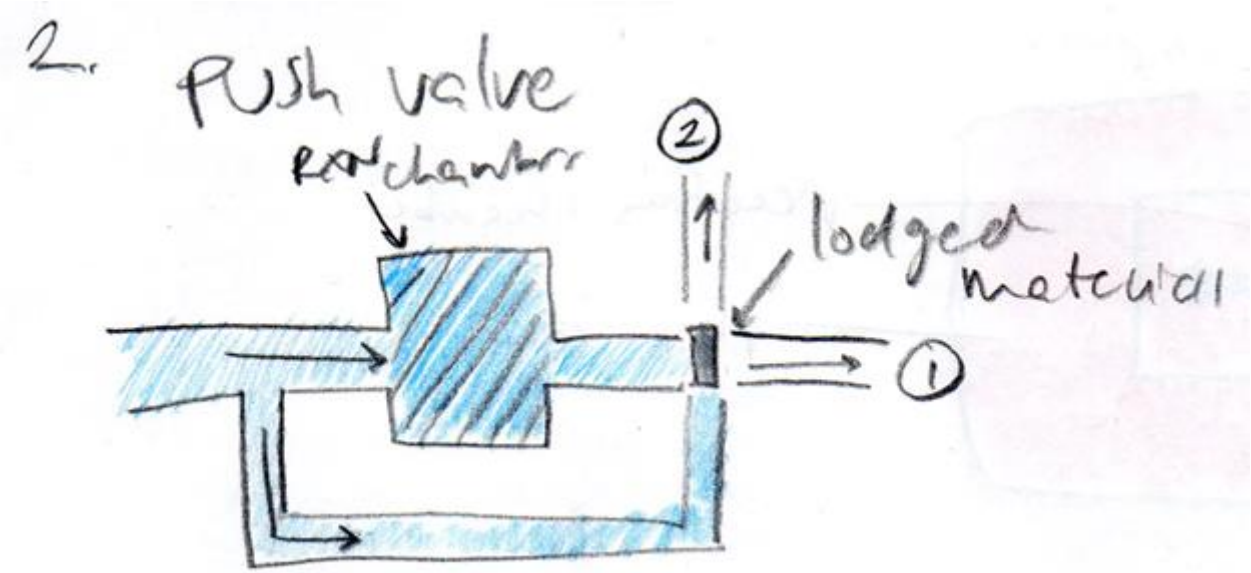

Figure D.6: A drawing of a design for a tested capillary value. (Drawn by Andy Ly)

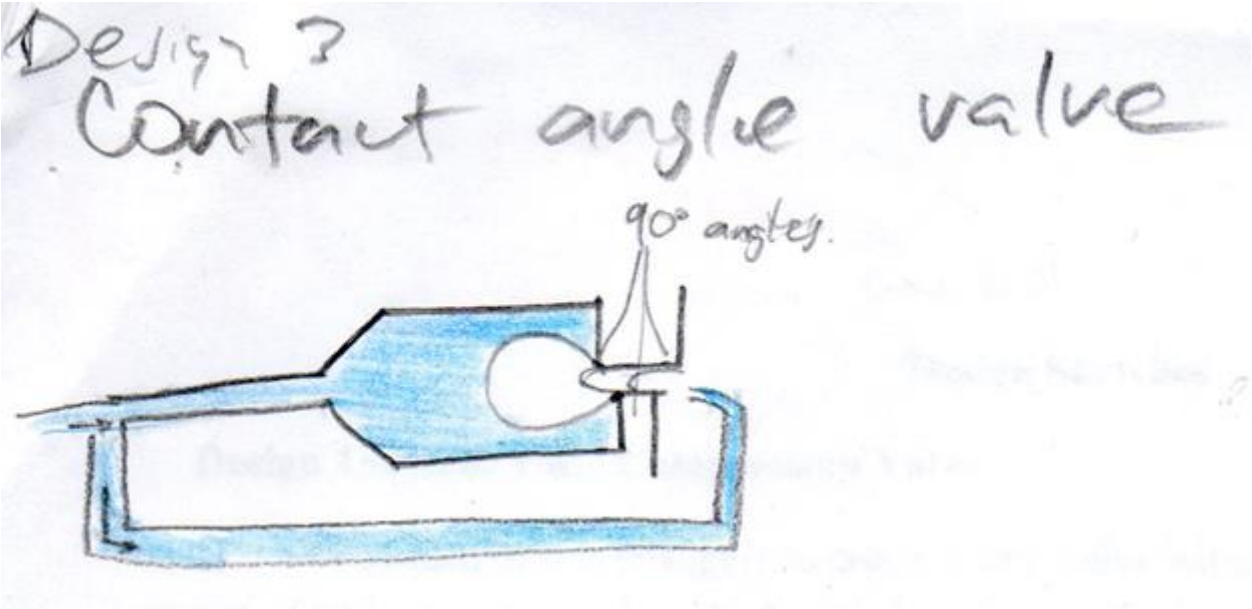

Figure D.7: A drawing of the design for a tested capillary value. (Drawn by Andy Ly)

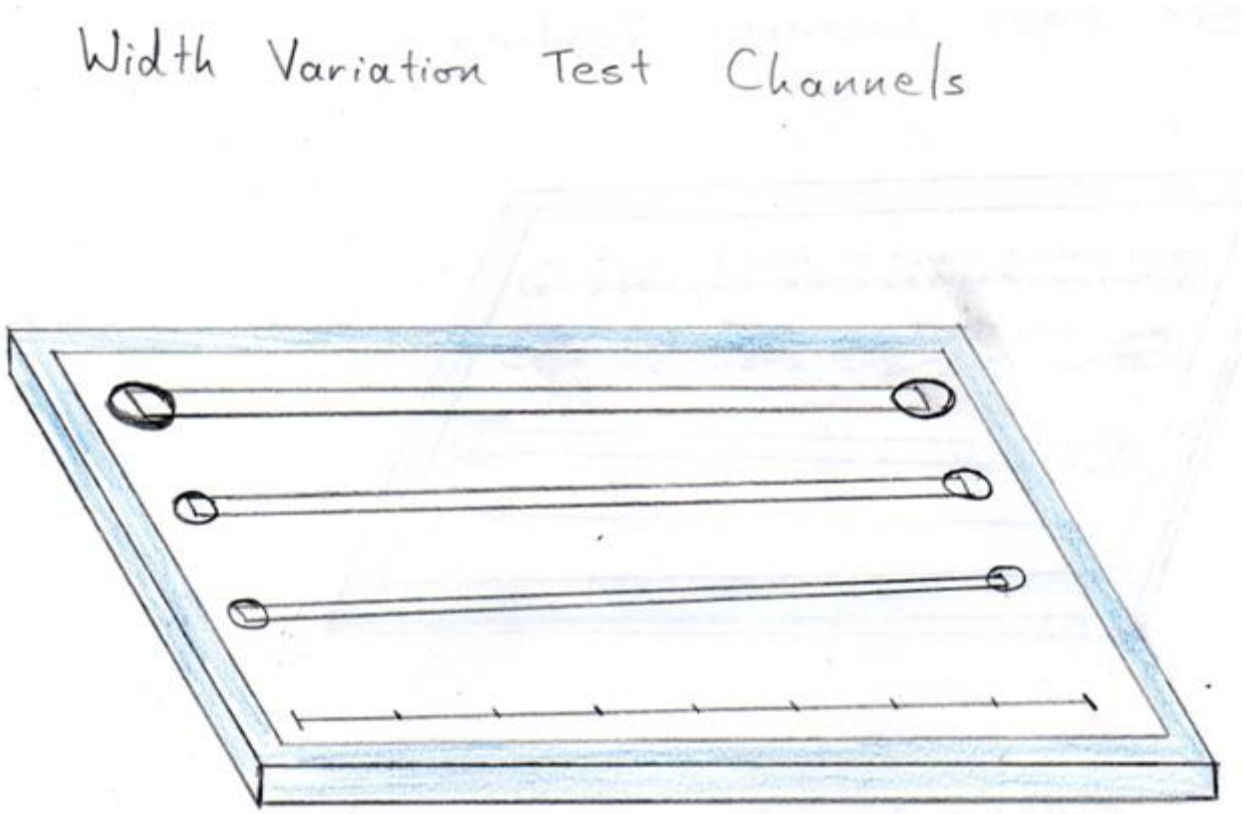

Figure D.8: A drawing of some of the channels designed to test the speed of the flow through the microchannels. (Drawn by Andres Maldonado-Liu)

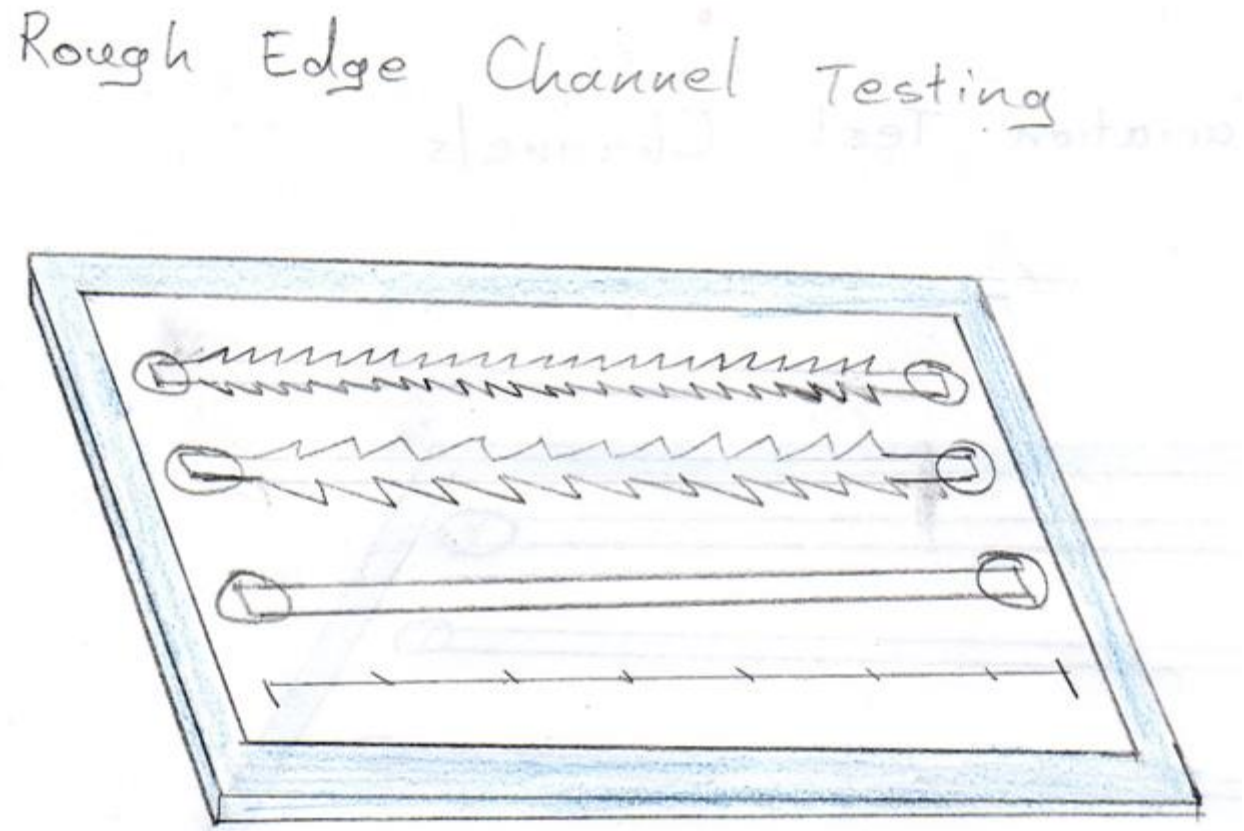

Figure D.9: A drawing of some of the channels designed to test if edges could slow down the flow in the microchannels. (Drawn by Andres Maldonado-Liu)

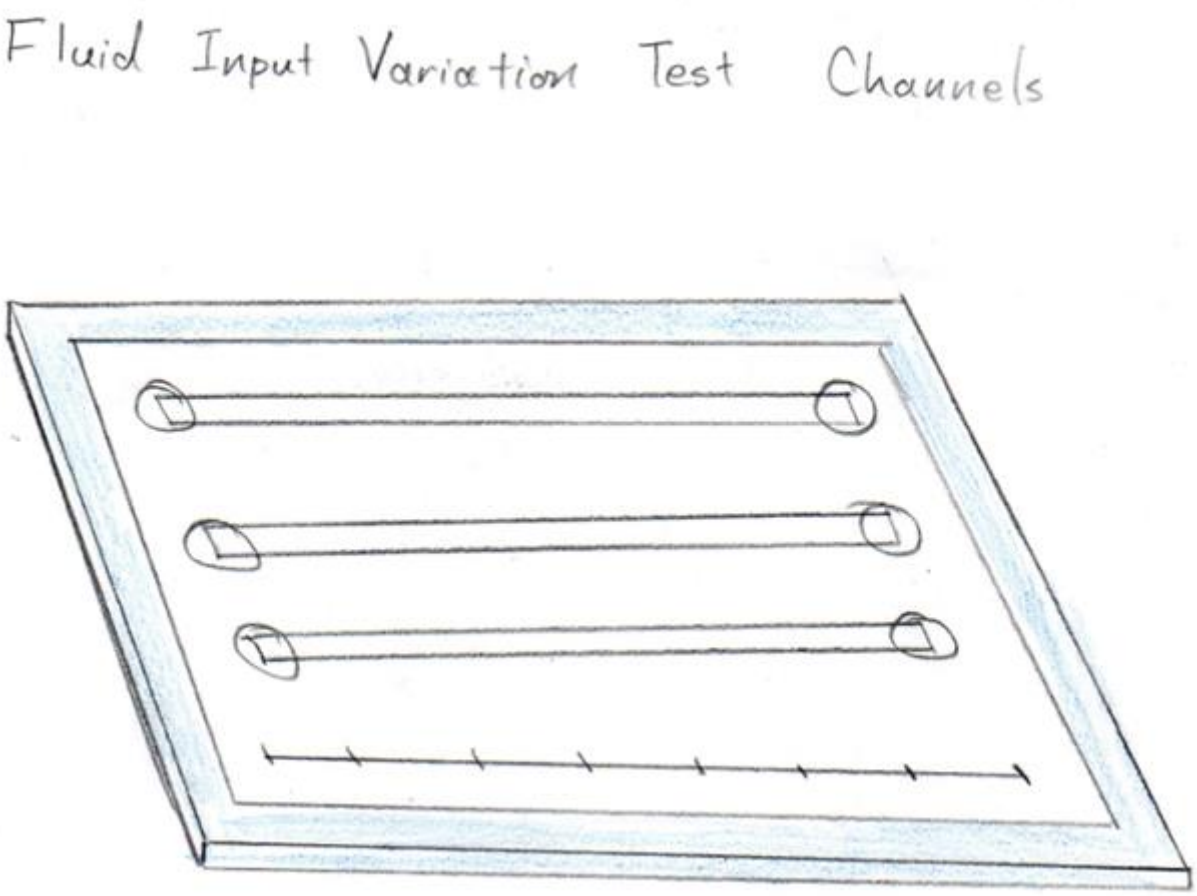

Figure D.10: A drawing of some of the channels designed to test if fluid variation would affect the flow. (Drawn by Andres Maldonado-Liu)

Appendix E: Timelines

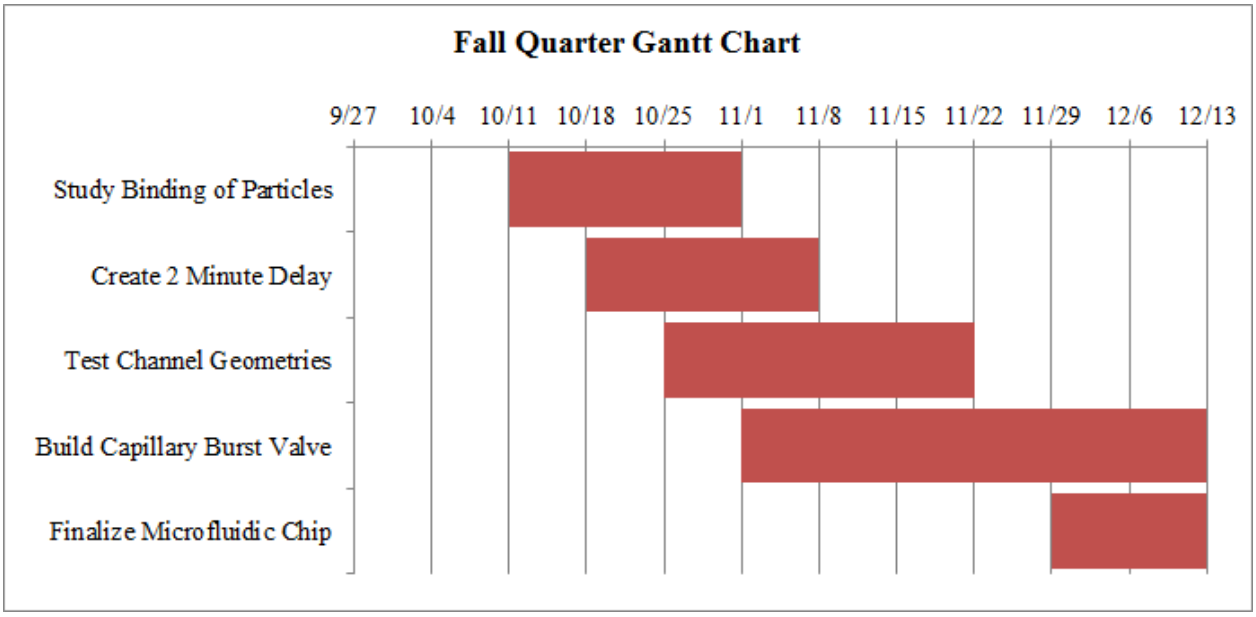

Figure E.1: The Gantt Chart that was followed for the Fall Quarter.

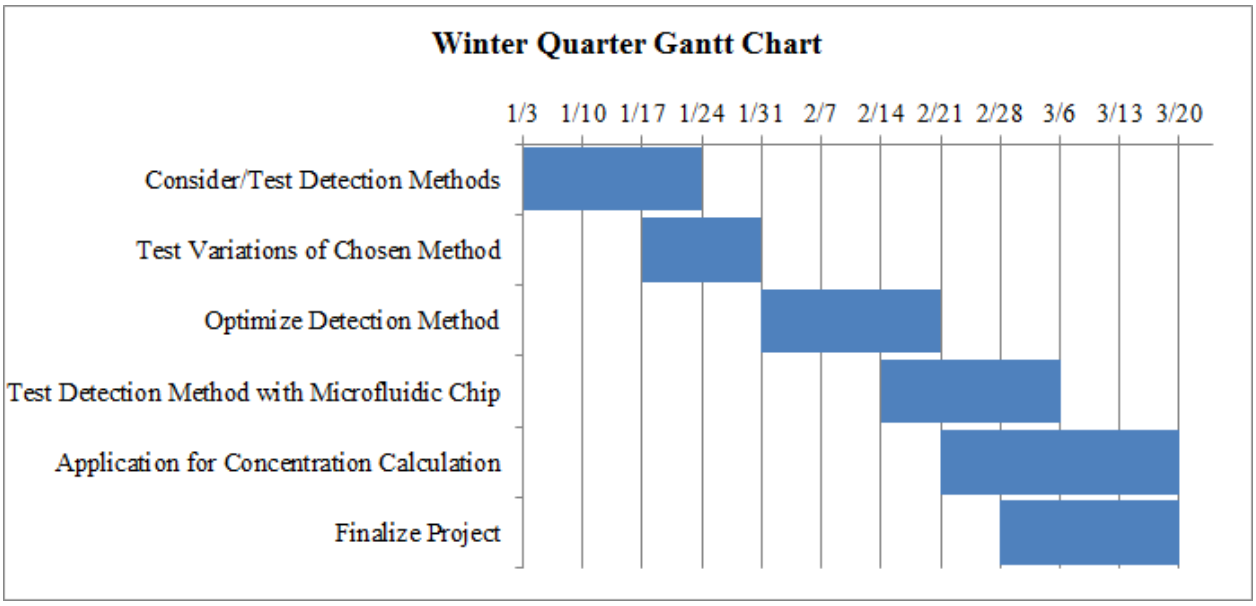

Figure E.2: The Gantt Chart that was followed during Winter Quarter.

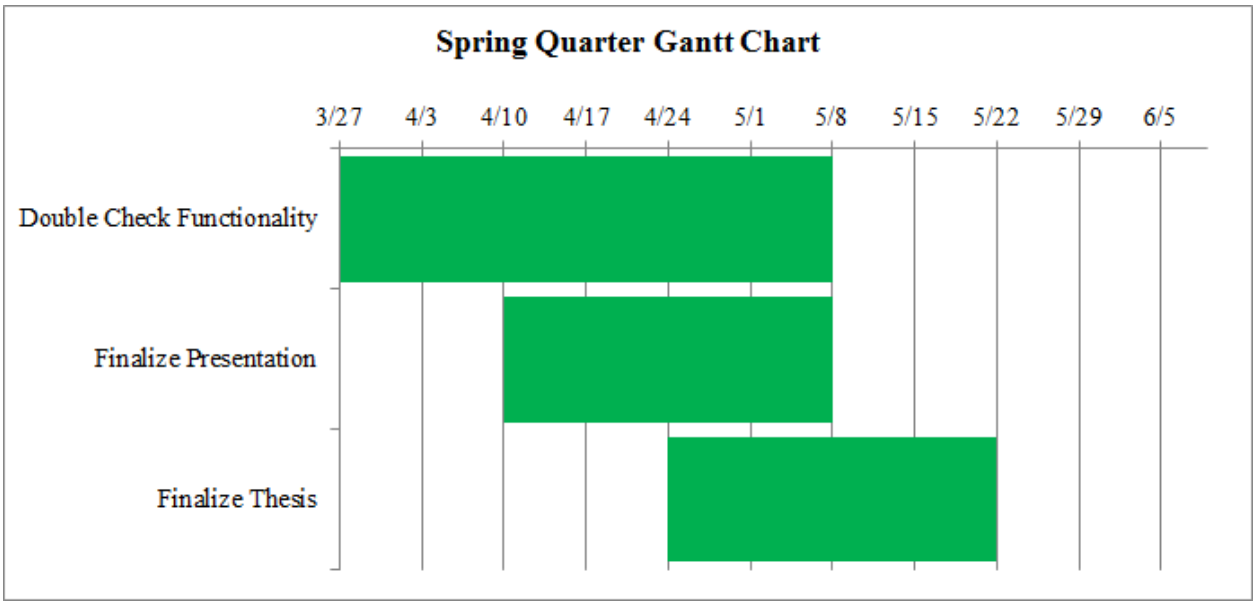

Figure E.3: The Gantt Chart that was followed during Spring Quarter.

Appendix F: Budget Spreadsheet

Table F.1: The anticipated costs for the project.

Item	Cost Per Unit ($)	Amount	Cost ($)	Notes
Craft Cutter	1100.00	1	1100.00	
ARFLOW 93049 Adhesive Roll	0.00		0.00	Free for research purposes
E. coli Detection Kit	120.00	3	360.00	
Glass slides	0.10	200	20.00	
Lysogeny Broth	90/kg	2	180	
			TOTAL COST ($)	
			1660.00	

Appendix G: Customer Needs Analysis

The following table shows the answers to the questions that were asked in the customer needs questionnaire.

Table G.1: The answers to the questions in the customer needs questionnaire, as inferred from 2 published articles [1, 2].

Answers to Customer Needs Questions Found in the Articles		
Question	Article 1 [1]	Article 2 [2]
Is there a need for a portable, contamination detector for water in developing countries?	Yes, many of the deaths seen particularly in South Africa are the effects from consuming contaminated water.	Yes, there are very few appropriate point-of-care diagnostic technologies for developing countries.
How would our product be used? In what situations?	The product would be used to check the contamination of water before using it.	The product would help to check water before consumption and could possibly be modified to help detect other diseases.
Have there been previous detection devices made? What were some of the problems?	Yes, but they had long assay times, required technical training, and were costly.	Yes, the current diagnostic tests are too expensive. require laboratory facilities, and are not sufficiently effective
What are the biggest challenges for providing healthcare products to developing countries?	Scarcity of running water, few reliable electrical services, lack of training, and no advanced technology in the area.	Inadequate funding, a poor understanding of needed tests, lack of protocols, and lack of access to markets for purchase of products.
What are the most desirable qualities of a contamination detection device?	Low cost, rugged, accurate, and reliable.	Quick detection time, accessibility, simple, reliable, and cheap.
What could be improved upon our proposed design of previous detection methods?	The design project's detection method should not use any advanced technology, or technology in general.	Having a product that could detect multiple diseases with no need for a laboratory to perform the tests.
Which is more important: a quick detection time, a low cost, or a small size?	All are fairly important, but having a low cost would allow more people to obtain the product.	A quicker detection time. Health care clinics in developing countries will mix-up results if they have them for too long.

Appendix H: Inputs and Outputs

The following table shows the anticipated inputs and outputs of the system.

Table H.1: The anticipated inputs and outputs of the system.

Inputs	Outputs
Sample Water	Contamination Concentration of *E. coli*
Latex Particles	Sample Water with Latex Particles

Appendix I: Experimental Data

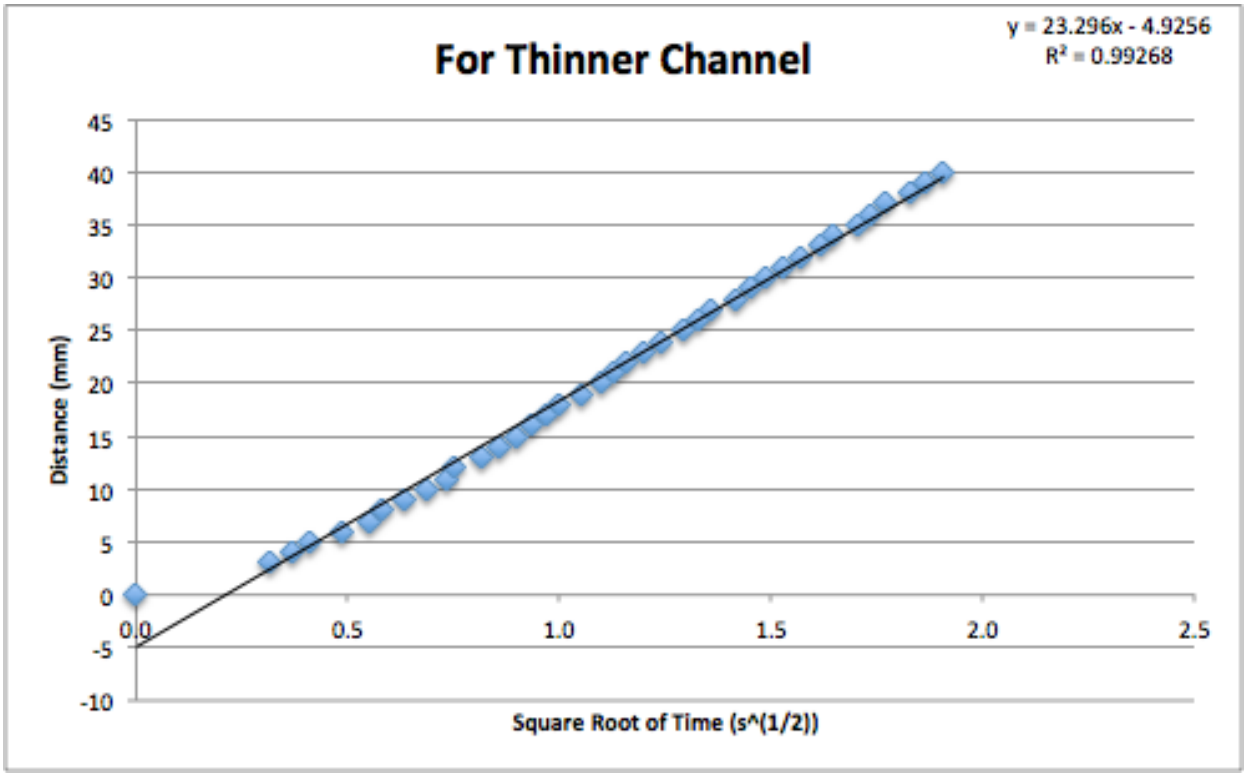

Figure I.1: A square root of time vs. distance chart used to validate the assumption that the distance traveled is proportional to the square root of time elapsed. This analysis was performed by filming a straight channel flow and recording the distance traveled by the flow as well as the time elapsed in the flow. Two channels were tested and this was the thinner of the two.

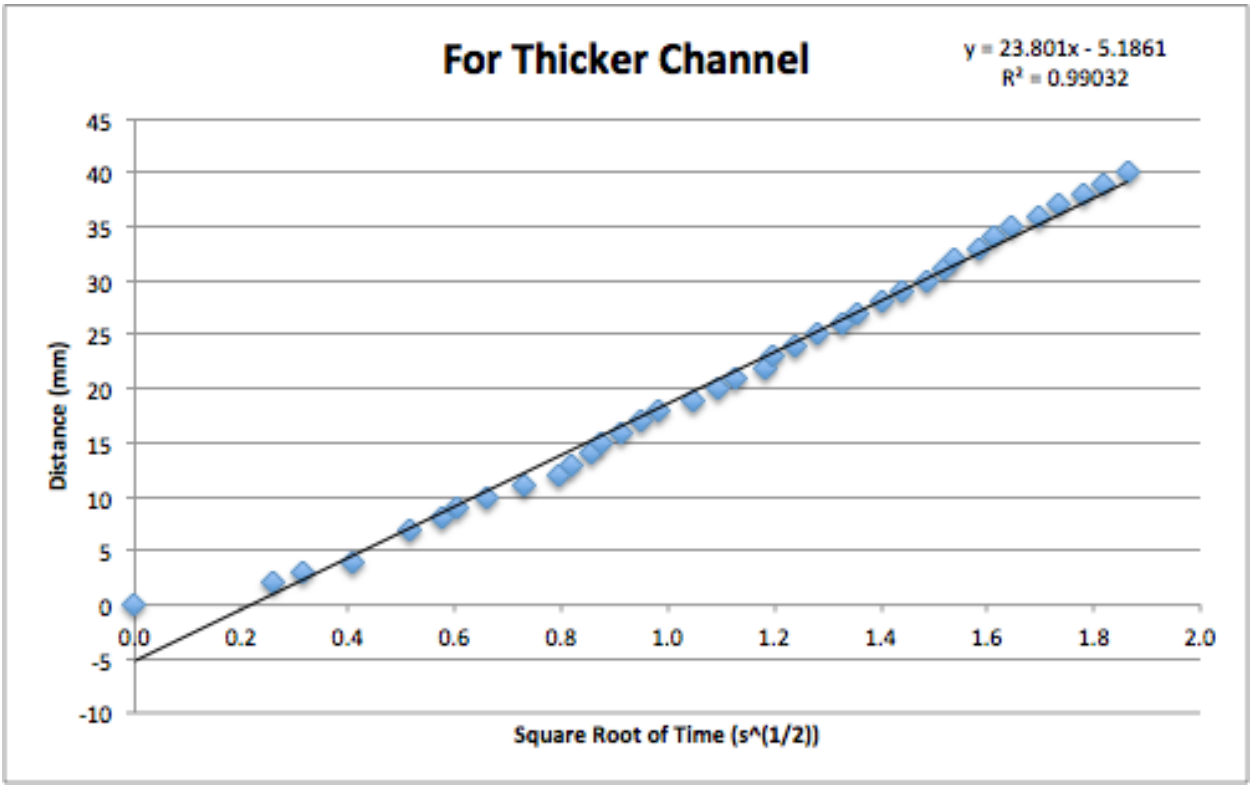

Figure I.2: A square root of time vs. distance chart used to validate the assumption that the distance traveled is proportional to the square root of time elapsed. This analysis was performed by filming a straight channel flow and recording the distance traveled by the flow as well as the time elapsed in the flow. Two channels were tested and this was the thicker of the two.

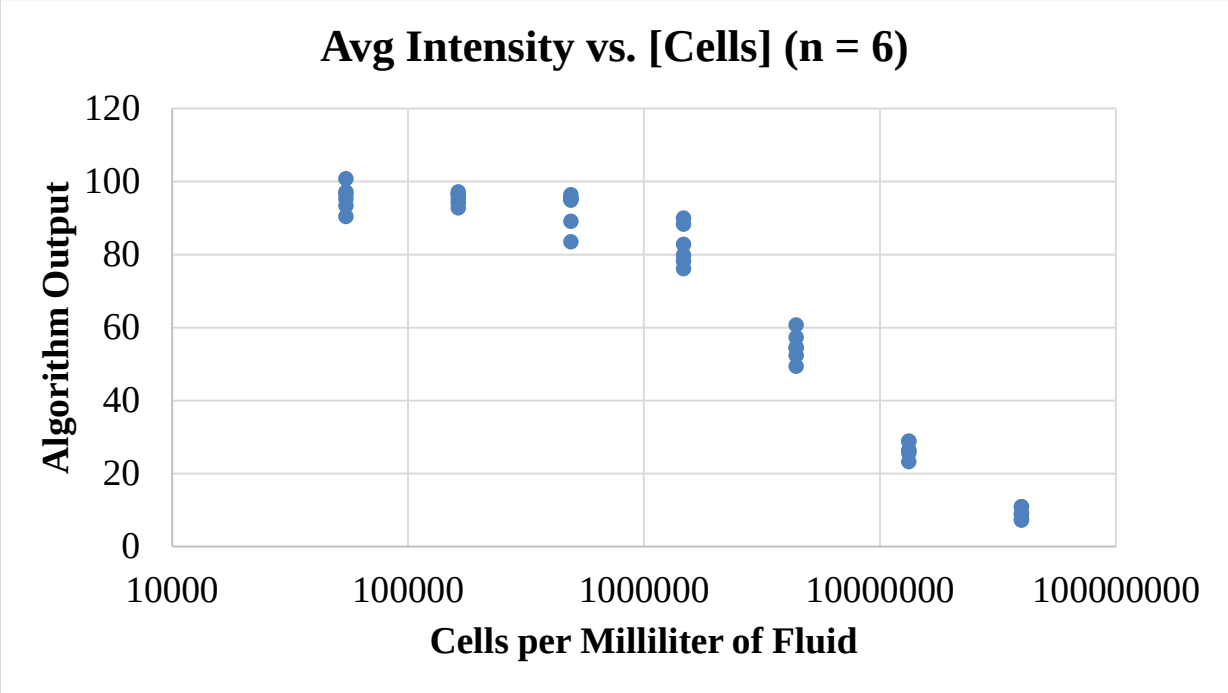

Figure I.3: Experimental data obtained to create the general correlation curve for the detection system.

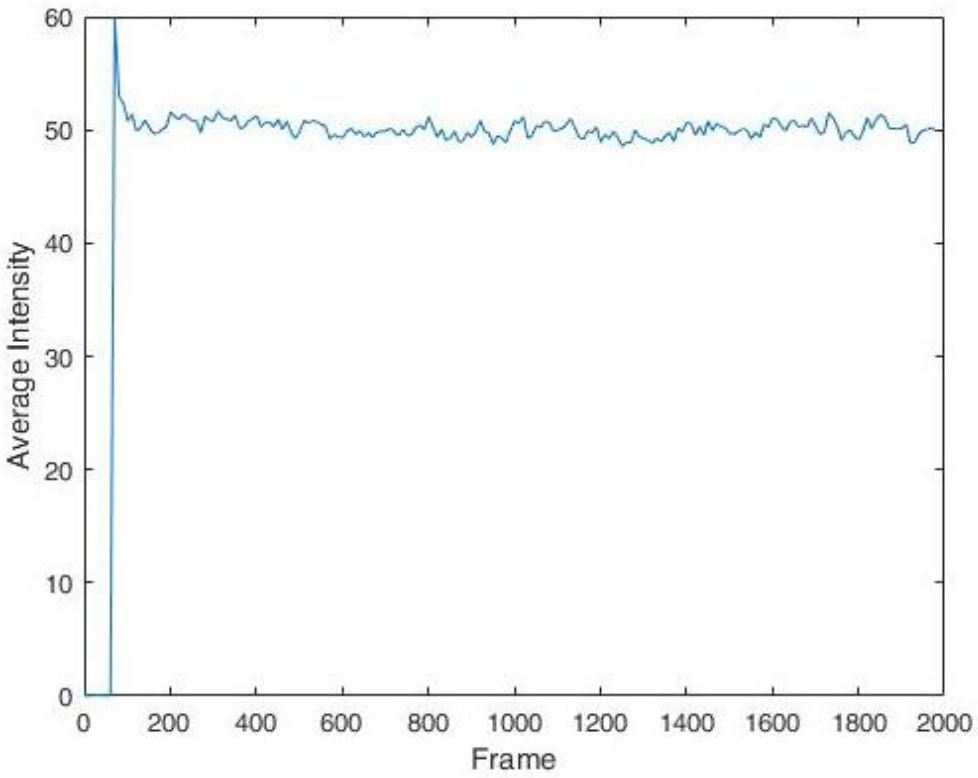

Figure I.4: A sample of the output signal from the algorithm used to find the concentration of a sample fluid. This figure shows the average pixel intensity found in the video at each frame of the video.

Appendix J: Manufacturer Information

Adhesives Research, Inc.

400 Seaks Run Road
PO Box 100
Glen Rock, PA 17327
Phone: +1 (717) 235-7979
Toll-free: +1 (800) 445-6240
Fax: +1 (717) 235-8320

Ted Pella, Inc.

P.O. Box 492477, Redding, CA 96049-2477
Telephone: 530-243-2200; 800-237-3526
Fax: 530-243-3761
Email: sales@tedpella.com (USA)
Email: isales@tedpella.com (International)

Appendix K: Code

```
close all
clear all
clc
vid_file = input('Enter File \n', 's');
vid_file = [vid_file '.mp4'];
% file = dir(vid_file);
% [n, m] = size(fldr);

vid = VideoReader(vid_file);
vid_length = floor(vid.Duration*vid.FrameRate);

% Baseline average intensity calculations
bl_org_pic = read(vid,1); % Read baseline image
bl_gray_pic = rgb2gray(bl_org_pic); % Convert baseline image to grayscale
pic_size = numel(bl_gray_pic);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%
% SECTION I: take pictures towards the end and use them to find where
% the channel is.

random_frame_with_fluid1 = double(rgb2gray(read(vid,vid_length*20/24)));
random_frame_with_fluid2 = double(rgb2gray(read(vid,vid_length*18/24)));
random_frame_with_fluid3 = double(rgb2gray(read(vid,vid_length*16/24)));
random_frame_with_fluid4 = double(rgb2gray(read(vid,vid_length*14/24)));
random_frame_with_fluid5 = double(rgb2gray(read(vid,vid_length*12/24)));

initial_intense_avg = double((random_frame_with_fluid1 + random_frame_with_fluid2 +
random_frame_with_fluid3 + random_frame_with_fluid4 + random_frame_with_fluid5)/5);
initial_intense_std = sqrt(((random_frame_with_fluid1-initial_intense_avg).^2 +
(random_frame_with_fluid2-initial_intense_avg).^2 + (random_frame_with_fluid3-
initial_intense_avg).^2 + (random_frame_with_fluid4-initial_intense_avg).^2 +
(random_frame_with_fluid5-initial_intense_avg).^2)/5);
initial_intense_std_int = uint8(initial_intense_std);

std_thresh = 1; %We cycle it now.
org_initial_intense_std_int = initial_intense_std_int;

No_concentration_thresh = floor(0.00012056*pic_size); %0.012056% of the pixels
```

```
for ii = 1:253
   std_thresh = std_thresh + ii;
   initial_intense_std_int = org_initial_intense_std_int;
   initial_intense_std_int(initial_intense_std_int<std_thresh) = 0;
   initial_intense_std_int(initial_intense_std_int>0) = 255;
   lit_pixels = numel(initial_intense_std_int(initial_intense_std_int>0));
   if lit_pixels <= floor(0.041*pic_size) && lit_pixels >= No_concentration_thresh %85000 and
250
      break
   end
end

% Arbitrary, but lowest concentration so far shows 1615 pixels lit up.

if numel(initial_intense_std_int(initial_intense_std_int>0)) <= No_concentration_thresh
   error('ERROR: Device was unable to find agglutination due to E. Coli absence or small
concentration.');
end

figure(1) % Sanity Check: see what the computer sees before cropping.
imshow(initial_intense_std_int)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%
% SECTION II: take picture from last section and create the crop box.
% Picture gets flipped if necessary for polyfit, which is geared toward linear regressions in
% terms of x.

oaz = initial_intense_std_int == 255;
[row, col] = find(oaz);

if std(col)/std(row) >= 0.55 && std(row)/std(col) >= 0.55
   if std(col) > std(row)
      oaz(1:ceil(mean(row)-0.7*std(row)),:) = 0;
      oaz(ceil(mean(row)+0.7*std(row)):end,:) = 0;
      right_side = mean(col(col>vid.Width/2));
      left_side = mean(col(col<vid.Width/2));
      oaz(:,1:ceil(left_side)) = 0;
      oaz(:,floor(right_side):end) = 0;
   elseif std(col) <= std(row)
      oaz(:,1:floor(mean(col)-0.7*std(col))) = 0;
      oaz(:,floor(mean(col)+0.7*std(col)):end) = 0;
   else
      error('Problems finding the channel.')
   end
```

```
end

[row, col] = find(oaz);
figure(912)
imshow(oaz); % Shows the codes new view of the channel

if std(col) <= std(row) % If it sees Vertical Channel.
   P = polyfit(row,col,1);
   fitted_horizontal_channel_pic = P(1)*(1:vid.Height) + P(2);
   std_error = 0;
   for qw = 1:numel(col)
      std_error = std_error + abs(fitted_horizontal_channel_pic(row(qw))-col(qw)); % Here we
create the lines that will allow
   end
   std_error = std_error/(numel(col));
   error_line1_horizontal_channel_pic = P(1)*(1:vid.Height) + P(2) - 9*std_error/10; %us to
crop into the channel. These lines
   error_line2_horizontal_channel_pic = P(1)*(1:vid.Height) + P(2) + 9*std_error/10; %lie
inside the channel. ARBITRARY(80% STD)
   bl_gray_pic_flip = flipdim(bl_gray_pic',2); % Calculations will be performed on the flipped
picture
   %so we need the flipped baseline picture.

   bot_line = ceil(error_line1_horizontal_channel_pic); % Line points need to be whole numbers
to access matrix.
   top_line = floor(error_line2_horizontal_channel_pic);
   vertical_divide_left = ceil(mean(row) - 1.1*std(row)); %floor(vid.Height*0.15); % Arbitrarily
chosen. Just want to cut out the channel parts near the ends.
   vertical_divide_right = floor(mean(row) + 1.1*std(row)); %floor(vid.Height*0.85);
   vertical_divide_slit_left = ceil(mean(row) - 0.02*std(row));
   vertical_divide_slit_right = floor(mean(row) + 0.02*std(row));

   figure(2) % Critical figure. This shows where the crop was made.
   scatter(row,col)
   xlim([0 vid.Height])
   ylim([0 vid.Width])
   xlabel('row')
   ylabel('col')
   hold on
   Graph1 =
plot(1:vid.Height,error_line1_horizontal_channel_pic,'k',1:vid.Height,error_line2_horizontal_ch
annel_pic,'k',[vertical_divide_left vertical_divide_left],[0 vid.Width],'k',[vertical_divide_right
vertical_divide_right],[0 vid.Width],'k',[vertical_divide_slit_left vertical_divide_slit_left],[0
vid.Width],'k',[vertical_divide_slit_right vertical_divide_slit_right],[0
vid.Width],'k'); %row,fitted_flipped_pic,
```

```
    set(Graph1(1),'linewidth',2)
    set(Graph1(2),'linewidth',2)
    set(Graph1(3),'linewidth',2)
    set(Graph1(4),'linewidth',2)
elseif std(col) > std(row) % If it sees Horizontal Channel.
    P = polyfit(col,row,1);
    fitted_horizontal_channel_pic = P(1)*(1:vid.Width) + P(2);
    std_error = 0;
    for qw = 1:numel(row)
        std_error = std_error + abs(fitted_horizontal_channel_pic(col(qw))-row(qw)); % Here we
create the lines that will allow
    end
    std_error = std_error/(numel(row));
    error_line1_horizontal_channel_pic = P(1)*(1:vid.Width) + P(2) - 9*std_error/10; %us to crop
into the channel. These lines
    error_line2_horizontal_channel_pic = P(1)*(1:vid.Width) + P(2) + 9*std_error/10; %lie inside
the channel. ARBITRARY(80% STD)
    bl_gray_pic_flip = bl_gray_pic; % Calculations will be performed on the flipped picture
    %so we need the flipped baseline picture.

    bot_line = ceil(error_line1_horizontal_channel_pic); % Line points need to be whole numbers
to access matrix.
    top_line = floor(error_line2_horizontal_channel_pic);
    vertical_divide_left = ceil(mean(col) - 1.1*std(col));%ceil(min(col) + ceil(max(col)-
min(col))*0.15);
    vertical_divide_right = floor(mean(col) + 1.1*std(col));%floor(max(col) - ceil(max(col)-
min(col))*0.15);
    vertical_divide_slit_left = ceil(mean(col) - 0.02*std(col));
    vertical_divide_slit_right = floor(mean(col) + 0.02*std(col));

    figure(2) % Critical figure. This shows where the crop was made.
    scatter(col,row)
    ylim([0 vid.Height])
    xlim([0 vid.Width])
    ylabel('row')
    xlabel('col')
    hold on
    Graph1 =
plot(1:vid.Width,error_line1_horizontal_channel_pic,'k',1:vid.Width,error_line2_horizontal_cha
nnel_pic,'k',[vertical_divide_left vertical_divide_left],[0 vid.Height],'k',[vertical_divide_right
vertical_divide_right],[0 vid.Height],'k',[vertical_divide_slit_left vertical_divide_slit_left],[0
vid.Height],'k',[vertical_divide_slit_right vertical_divide_slit_right],[0
vid.Height],'k'); %row,fitted_flipped_pic,
    set(Graph1(1),'linewidth',2)
    set(Graph1(2),'linewidth',2)
    set(Graph1(3),'linewidth',2)
```

```
    set(Graph1(4),'linewidth',2)
else
    error('ERROR: There was an error in the channel detection process.');
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%
% SECTION III: this section quantifies the pictures that were presented.
% Takes the lines created and only finds the intensity and area ratio
% between them.

small_thresh = 10; % This might be able to be deleted. Just filters out a little noise from
background.
%should it be 0, 5, 10, 20, 40?
area_thresh = 50; % experiementally found. Allows one to see which side of the curve were on.
area_thresh_slit = 100;
counter = 1;
display(counter);

take_it_this_many_frames = 30;

for j = 1:take_it_this_many_frames:vid_length-take_it_this_many_frames
    frame = rgb2gray(read(vid,j)); % Finds the difference in the initial picture and each frame.
    if std(col) <= std(row)
        frame = flipdim(frame',2);
    end
    intensity_difference_Matrix = (bl_gray_pic_flip - frame) - small_thresh;
    intensity_difference_Matrix(intensity_difference_Matrix<0) = 0;
    intensity_difference_Matrix(intensity_difference_Matrix>0) =
intensity_difference_Matrix(intensity_difference_Matrix>0) + small_thresh;

    added_intensity = 0; % Counts only the stuff in the crop box.
    added_intensity_slit = 0;
    total_pixels_in_crop = 0;
    total_pixels_in_crop_slit = 0;
    number_dark_pixels = 0;
    number_dark_pixels_slit = 0;
    for i = 1:vertical_divide_right-vertical_divide_left
        total_pixels_in_crop = total_pixels_in_crop +
numel(intensity_difference_Matrix(bot_line(i-1+vertical_divide_left):top_line(i-
1+vertical_divide_left),i-1+vertical_divide_left));
        added_intensity = added_intensity + sum(intensity_difference_Matrix(bot_line(i-
1+vertical_divide_left):top_line(i-1+vertical_divide_left),i-1+vertical_divide_left));
```

```
        number_dark_pixels = number_dark_pixels +
numel(intensity_difference_Matrix(intensity_difference_Matrix(bot_line(i-
1+vertical_divide_left):top_line(i-1+vertical_divide_left),i-1+vertical_divide_left)>area_thresh));
        if i >= vertical_divide_slit_left-vertical_divide_left && i <= vertical_divide_slit_right-
vertical_divide_left
            total_pixels_in_crop_slit = total_pixels_in_crop_slit +
numel(intensity_difference_Matrix(bot_line(i-1+vertical_divide_left):top_line(i-
1+vertical_divide_left),i-1+vertical_divide_left));
            added_intensity_slit = added_intensity_slit +
sum(intensity_difference_Matrix(bot_line(i-1+vertical_divide_left):top_line(i-
1+vertical_divide_left),i-1+vertical_divide_left));
            number_dark_pixels_slit = number_dark_pixels_slit +
numel(intensity_difference_Matrix(intensity_difference_Matrix(bot_line(i-
1+vertical_divide_left):top_line(i-1+vertical_divide_left),i-
1+vertical_divide_left)>area_thresh_slit));
        end
    end
    avg_intensity(counter) = added_intensity/total_pixels_in_crop;
    avg_intensity_slit(counter) = added_intensity_slit/total_pixels_in_crop_slit;
    area_ratio(counter) = number_dark_pixels/total_pixels_in_crop;
    area_ratio_slit(counter) = number_dark_pixels_slit/total_pixels_in_crop_slit;

    counter = counter + 1;
    display(counter);
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%
% SECTION VI: find your data. Shows the intensity and area ratio per frame
% and allows for you to pick the frame of where to begin finding the
% average and stuff.

%% Figures and Results

figure(4)
plot(1:take_it_this_many_frames:vid_length-take_it_this_many_frames, avg_intensity)
xlabel('frame')
ylabel('difference')

figure(5)
plot(1:take_it_this_many_frames:vid_length-take_it_this_many_frames, area_ratio)
xlabel('frame')
ylabel('area ratio')

figure(6)
```

```
plot(1:take_it_this_many_frames:vid_length-take_it_this_many_frames, avg_intensity_slit)
xlabel('frame')
ylabel('slit difference')

figure(7)
plot(1:take_it_this_many_frames:vid_length-take_it_this_many_frames, area_ratio_slit)
xlabel('frame')
ylabel('slit area ratio')

min_ind = input('Enter post-flow frame \n');
min_ind = ceil((min_ind/take_it_this_many_frames) + 1);

avg_intensity_dummy = avg_intensity;


tot_intensity_average = mean(avg_intensity(min_ind:end));
display(tot_intensity_average); % Average of all average intensity values
s_dev_intensity = std(avg_intensity(min_ind:end));
display(s_dev_intensity);

area_ratio_average = mean(area_ratio(min_ind:end));
display(area_ratio_average);
s_dev_area = std(area_ratio(min_ind:end));
display(s_dev_area);

tot_intensity_average_slit = mean(avg_intensity_slit(min_ind:end));
display(tot_intensity_average_slit);
s_dev_intensity_slit = std(avg_intensity_slit(min_ind:end));
display(s_dev_intensity_slit);

area_ratio_average_slit = mean(area_ratio_slit(min_ind:end));
display(area_ratio_average_slit);
s_dev_area_slit = std(area_ratio_slit(min_ind:end));
display(s_dev_area_slit);


fluid_intensity = abs(avg_intensity(min_ind:end) - tot_intensity_average);
fluid_intensity(fluid_intensity<0) = 0;
fluid_intensity = sum(fluid_intensity);

display(fluid_intensity);
```

Appendix L: Presentation Slides

The Senior Design Conference presentation slides can be found on the following pages.

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Microfluidic *E. coli* Detection

Team BakPak

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Introduction

Team Members

- Andres Maldonado: MECH
- Andy Ly: MECH
- Kyle Pietrzyk: MECH
- Scott Fukuoka: BIOE

Faculty Advisors

- Dr. On Shun Pak: MECH
- Dr. Unyoung Kim: BIOE

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Problem Statement

- In developing countries, 80% of illnesses linked to unsanitary water.
- A low cost diagnostic system for communities to check their water for contaminants.
- Avoid life-threatening bacteria, such as *E. coli* O157:H7.

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Customer Needs: The ASSURED Standard

Affordable
Sensitive
Specific
User-Friendly
Rapid and Robust
Equipment-Free
Delivered

World Health Organization

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Product Design Specifications

	Filter's Fast Detection Kit [1]	Salmonella Detection Research[2]	Traditional Agglutination Test[3]	Our Product Targets
Detection time	48 hrs	10 mins	3 mins	<10 mins
Cost per test	$10.00	<$1.00	<$1.00	<$1.00
Cost for equipment	$0.00	High (Optical system)	$0.00	$8
Portable	✓	✗	✓	✓
Quantifiable	✗	✓	✗	✓
Specific	✗	✓	✓	✓
Lower Detection Limit	High	Low (10^1 cells/mL)	High (> 10^6 cells/mL)	High (10^6 cells/mL)

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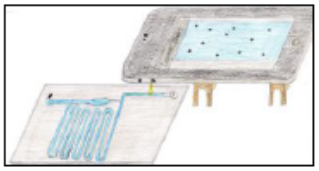
Our Approach

Two component product

- **Microfluidic chip**
  - Mixes detection particles with *E. coli*
  - Provides a platform for detection
- **Detection method**
  - Determines concentration of *E. coli* within fluid



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Microfluidic Chip

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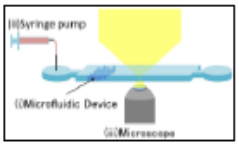
Traditional Microfluidic Devices

- Characterized by
  - Small fluid volumes (microliters)
- Typically made of PDMS (Polydimethylsiloxane)
  - Transparent
  - Low cost
  - Special equipment
- Fluid flow is driven by external sources



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Capillary Driven Flow

- Surface tension at interface between solids and fluids
- On a micro-scale, capillary forces can drive a fluid
- Eliminates the need for external pumping

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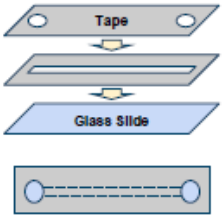
Building the Microfluidic Platform

- Layered construction
  - Simplifies manufacturing
- Hydrophilic adhesive tape
  - Low contact angle
  - Drives flow
  - Transparent material



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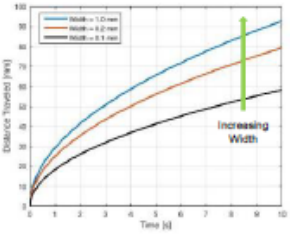
Microfluidic Chip Design: Channel Width

- Flow speed
  - Low speed
    - Long detection time
  - High speed
    - Poor resolution image
- Washburn model

$$L^2(t) = \left[\left(1 - 0.63\frac{h}{w}\right)\frac{h\gamma\cos\theta}{3\mu}\right]t$$



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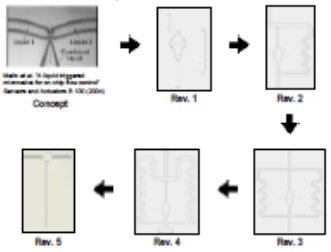
Microfluidic Chip Design: Initial Concept

- Automatic valve
  - Capillary burst valve
- Complex design
  - Requires precision manufacturing



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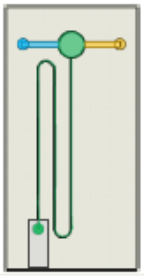
Microfluidic Chip Design: Final Concept

- Manual value
  - Scotch tape valve
  - User manually removes tape to open valve
- Layer material
  - Top layer: hydrophilic adhesive tape
  - Middle layer: hydrophilic adhesive tape
  - Bottom layer: glass slide



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Detection Method

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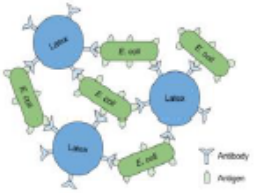
Latex Particle Agglutination

- Specific binding between antibody and antigen
- Latex particles functionalized with antibodies for *E. coli O157:H7*



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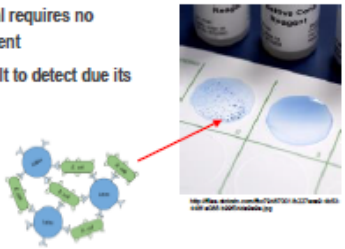
Detecting *E. coli*

- Amplified signal requires no special equipment
- *E. coli* is difficult to detect due its small size



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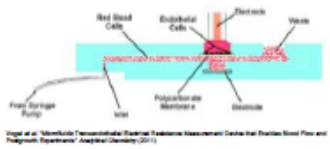
Four Possible Detection Methods

- Electrical Impedance Analysis (1)
  - Augment latex particles to alter electrical properties of fluid
    - Detect changes in capacitance or resistance
    - Use multimeter



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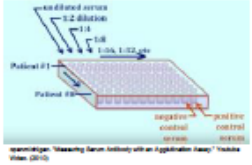
Four Possible Detection Methods

- Dilution Analysis (2)
  - Multiple well with different concentrations of latex particles
  - Measure agglutination pellet at bottom of well



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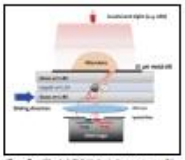
Four Possible Detection Methods

- **Light Diffraction Detection (3)**
  - Microlens to focus light through channel
  - Algorithm detects scattered light



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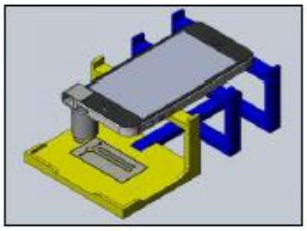
Direct Image Optical Analysis (4)

- Processes smartphone videos
- Algorithm automates analysis
- Concentration curve quantifies signal



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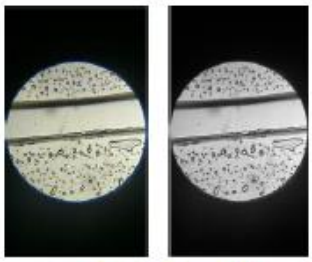
Optical Detection Algorithm

1. Select baseline frame
   - The video's first frame
2. Grayscale the video



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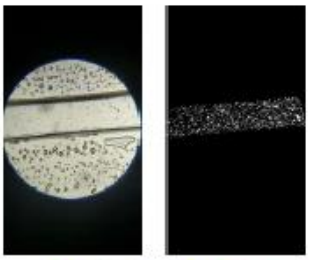
Optical Detection Algorithm (Cont.)

3. Find the channel in the video
   - Look for changing pixels
   - Use statistical analysis



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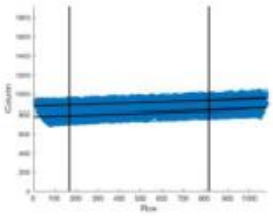
Optical Detection Algorithm (Cont.)

4. Crop frames to channel
   - Eliminates noise in frames
5. Measure pixel intensity for each modified frame



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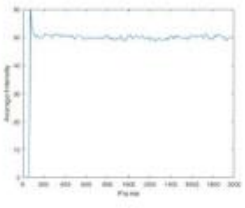
Optical Detection Algorithm (Cont.)

6. Subtract baseline intensity from measurements
   - Eliminates background noise
7. Calculate average pixel intensity per frame
8. Interpolate concentration from intensity curve



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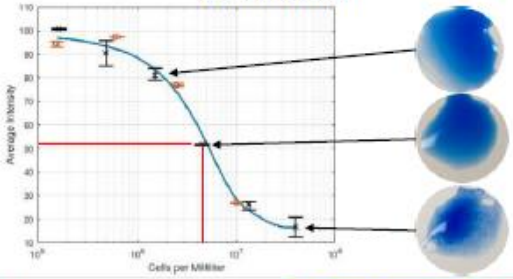
Correlation Curve for *E. coli* Concentration



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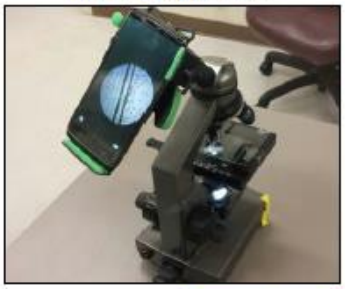
Current Apparatus



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Product Design Specifications

	Target Values	Actual Values
Detection time	<10 mins	<10 mins
Cost per test	<$1.00	~$1.00
Required Equipment	Smartphone and lens	Smartphone and lens
Cost for equipment	$8 [Smartphone not included]	$150.00 (Magnification lens) [Smartphone not included]
Portable	✔	○
Quantifiable	✔	✔
Specific	✔	✔
Lower Detection Limit	10^5 cells/mL	~10^5 cells/mL

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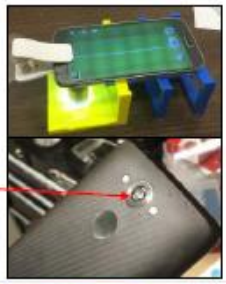
Work in Progress

- Improve the code
  - Increase accuracy
- Add vibration mixing
- Improve the stand
  - Fewer parts
  - Add enclosure
- Use a PDMS lens



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Conclusion

- Goal
  - Develop a low cost platform for *E. coli* detection in fluid samples
- Delivered Product
  - Microfluidic chip with detection method
- Further Developments
  - Add a water filter
  - Detect other pathogens

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Acknowledgements

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- Dr. Unyoung Kim

Special Thanks
- Dr. Tsung-Feng Wu
- Professor Robin Everest
- Adhesives Research
- SCU School of Engineering

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Questions?

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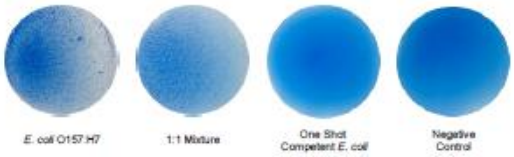
Specificity Test



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PDMS Lens

- Greatly reduces cost
  - Replaces microscope
- Portable
- Simple to make
  - Made on a hot plate

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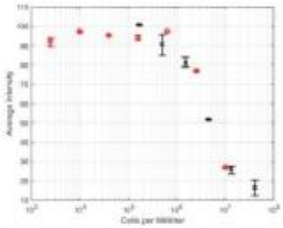
Full Concentration Curve

- Curve levels out at low concentrations
  - Reveals lower detection limit



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Chip Fabrication

- Vinyl Cutter was used
  - GraphTec Cutting Plotter CE6000-40
  - Fast and precise

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References

[1] FiltersFast
https://www.filtersfast.com/water-test-kit.asp?kpid=WS-425B&fsrc=G1F5F&gclid=CLL3v4bu48gCFZSFfgodCX8HkA

[2] Christopher F. Fonczek, David J. You, Jeong-Yeol Yoon. "Single-pipetting microfluidic assay device for rapid detection of Salmonella from poultry package." Biosensors and Bioelectronics 40 (2013): 342-349.

[3] Hardy Diagnostics
https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/EcoliPRO0157.htm