Rapid Plasma Separator

Final Report

Written by:

Sydney Bernstein, Morgan Perlman, and Sanjana Sharma

Collaborators:

Jane Choi and Jeannette Wu

Prepared For:

David Kelso, PhD

Mark Fisher

Northwestern
McCORMICK SCHOOL
OF ENGINEERING

Executive Summary

With the largest HIV epidemic in the world, South Africa has 7.7 million people living with HIV.[1] Thus, it is crucial that patients have easy access to efficient viral-load testing. The current laboratory practice in South Africa takes several weeks or months for patients to receive their tests; in fact, 50% of patients never receive their results.[2]

The client has therefore requested a method of separating plasma that will enhance an existing point-of-care (POC) system. A device will collect whole blood from a finger prick and ultimately isolate a minimum of 70 µL of plasma. Furthermore, it must be compatible with a single-sample centrifuge to separate blood into its components: plasma, white blood cells, and red blood cells. The isolated plasma will later be combined with a buffer solution in order to fulfill the 1 mL plasma requirement of the existing technology, the Cepheid GeneXpert. The cartridge containing the plasma and buffer will be used in the GeneXpert to test for HIV-1 Viral Load.

The team designed a blood collection device that relies on centrifugation and fluid balance to expel around 130 mL of blood into a sump while retaining approximately 70 µL of plasma. A centrifuge that holds a singular collection device was designed as well.

In addition to performing quantitative testing, two team members travelled to South Africa to perform user testing and observations in South African POC clinics. These members' insights were then incorporated in design iterations of both the centrifuge and collection device in order to best tailor the design towards the users needs.

---

[1] o

[2] Cepheid, “Cepheid Targets Development of a Point of Care HIV Viral Load Test From a Few Drops of Blood”, [https://www.cepheid.com/us/about-us/news-events/press-releases/250-cepheid-targets-development-of-a-point-of-care-hiv-viral-load-test-from-a-few-drops-of-blood], (October 8, 2019)

Table of Contents

List of Figures and Tables

Figures:

Tables

Abbreviations

ART: Antiretroviral Therapy

ELISA: Enzyme-Linked Immunosorbent Assay

HIV: Human Immunodeficiency Virus

LMIC: Low- and Middle-Income Country

NAT: Nucleic Acid Testing

PCR: Polymerase Chain Reaction

PLHIV: Person Living with HIV

POC: Point-of-Care

RT-PCR: Reverse Transcriptase Polymerase Chain Reaction

TB: Tuberculosis

UNAID: Joint United Nations Programme on HIV/AIDS

WHO: World Health Organization

1. Problem Overview

1.1 Problem Statement

Currently, viral load testing for HIV is a long process that involves drawing blood via venipuncture, transporting this sample to a central clinic, executing the test, and returning the results to the clinic and then finally the patient. The goal of this project is to design a device that will allow the testing to be performed at the point of care (POC), which would allow the patient to wait for their results at a local clinic. POC testing is crucial because it greatly diminishes the time frame in which patients receive their results. This allows them to receive appropriate treatment in a more timely and effective manner.

The first objective is to design a collection device for whole blood that can be centrifuged and isolate a minimum of 70 µL of plasma for testing. This device should be used with a finger stick because, according to the client, patients in South Africa prefer a finger stick to venipuncture for the amount of whole blood required, which is around 200 µL. Additionally, performing a finger stick requires less training and skill than venipuncture. After the collected blood has been centrifuged, the device should have a way of simply isolating the plasma and transferring it to the provided GeneXpert cartridge.

The second objective is to design a one-sample centrifuge that is compatible with the collection device and that is able to separate the whole blood into its components. This will help reduce the amount of time that a patient is waiting for their results and encourage them to stay and wait. The centrifuge should run off of the same voltage that is already present in the clinics, 220-240 volts, and exert enough acceleration to allow proper separation.

1.2 Requirements and Parameters

Dr. David Kelso is the Director of the Center for Innovation in Global Health Technology (CIGHT), and an Associate Professor of Biomedical Engineering at Northwestern University. His vision is to create a more efficient POC system that can be used to obtain quicker results at the patient's local clinic rather than a separate facility. This system includes a device that collects blood from a finger stick, as well as a centrifuge that can separate the plasma in the collected whole blood.

The blood collection device must:

- Be compatible with the one-sample centrifuge
- Allow transfer of plasma to the Cepheid's GeneXpert Cartridge
- Not leak blood during the centrifugation process
- Be compatible with a methodology for creating a plasma dilution

The centrifuge must:

- Run off of 220-240 V
- Exert enough acceleration to separate blood into its components
- Have the capacity for only one sample

The system (blood collection device and centrifuge) must:

- Remain functional in South African environmental conditions
- Isolate 70 µL of plasma with 95% of the leukocytes removed. This allows at least 40 copies/mL which is the limit of the Cepheid GeneXpert HIV-1 viral load test [3]

[3] Cepheid, "Cepheid Targets Development of a Point of Care HIV Viral Load Test From a Few Drops of Blood", [https://www.cepheid.com/us/about-us/news-events/press-releases/250-cepheid-targets-development-of-a-point-of-care-hiv-viral-load-test-from-a-few-drops-of-blood], (October 8, 2019)

1.3 Workflow

The following section discusses a further breakdown for each step within the system.

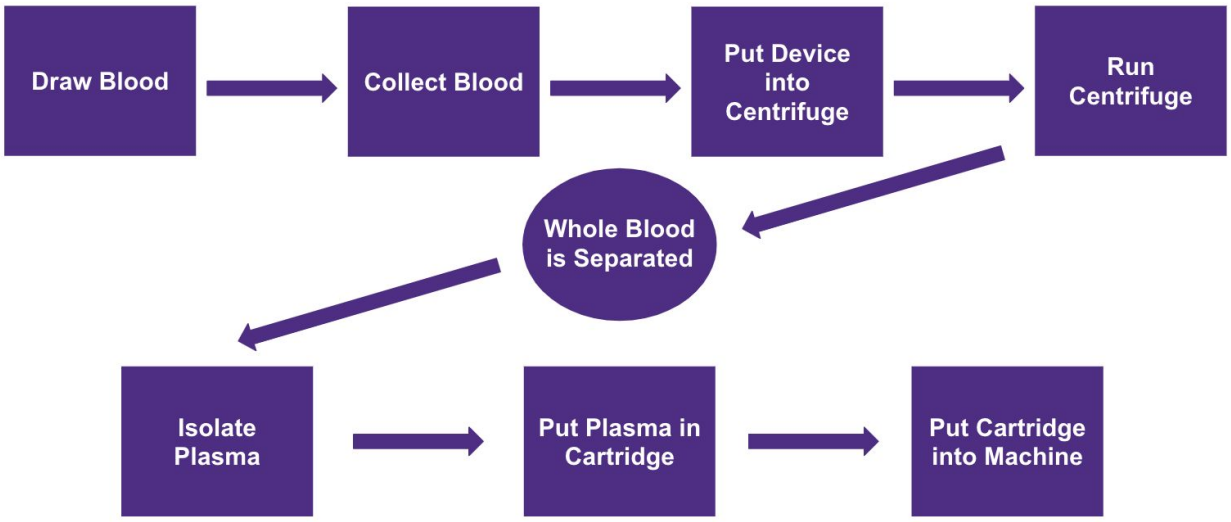


Figure 1: Workflow representing the steps involved in an ideal HIV diagnostic process

1.3.1 Draw Blood

The purpose of this step is to produce enough blood to be used in further processing and testing. The requirements from the client are a device that can produce 70 µL of plasma from whole blood obtained via a finger prick. Therefore, the only factor to consider for this step is the size of the lancet that will be cutting the finger; it must be able to penetrate deep enough to provide the requisite amount of blood.

1.3.2 Collect Blood

Once the patient's finger has been pricked and blood is flowing, the blood must be collected to use for testing. The parameters to consider include device geometry, surface

energy, number of entry points, and a vented end. The device geometry consists of aspects of its design such as diameter, length, and cross-sectional shape of the device. Surface energy is a critical factor since it plays a role in capillary action. According to the team's coach, Prof. Mark Fisher, using capillary action to collect blood is an easier technique than using gravity. The number of entry points for blood on the device can also vary from the traditional one. Lastly, the end of the device opposite the entry end must be vented in order to allow air to escape while blood is being collected. The team must come up with a method that stops the flow of blood but does not leak during the centrifugation step. When designing the collection device, the team should be cognisant of the requirements set by the client; it must hold 200 μL of blood, withstand forces applied by the centrifuge, and be able to sit securely in the centrifuge while it runs.

1.3.3 Put Device in Centrifuge and Run Centrifuge

After the blood has been collected into the collection device, the whole blood components must be separated by density. This step corresponds to the second aspect of the problem, the development of a centrifuge that runs on 220-240 volts that centrifuges one blood collection device. The centrifuge has four crucial factors: motor speed, spin duration, inertia, and radius of the rotor. The motor speed required for appropriate centripetal force depends on the radius of the rotor, as seen in Equation 1. The spin duration should be as short, keeping in mind that the patient at the POC facility should remain there for as little time as possible. The inertia of the centrifuge impacts how quickly the motor reaches its desired speed. The client, Dr. Kelso, has requested that the inertia be kept as low as possible, so that the motor can come up to speed quickly.

When designing the centrifuge, there are three hard requirements set by the client. The first requirement is that the centrifuge needs to run on 220-240 volts, the same voltage that the current POC facilities use for the Cepheid GeneXpert HIV-1 viral load assays. The next requirement is that the centrifuge should be as small as possible. This requirement will lead us to the radius of the rotor and then, in turn, the motor speed for the desired G-force. The last requirement is the centrifuge should only have the capacity for one blood sample.

1.3.4 Isolate Plasma and Put Plasma into Test Cartridge

After centrifugation, the plasma should have 95% of the leukocytes and 100% of the erythrocytes removed. Next, the plasma needs to be completely isolated from the red and white blood cells. This can be done two ways, by either removing the plasma from the collection device, leaving behind just the red and white blood cells, or by removing the red and white blood cells so that only the plasma is left in the device. After isolation occurs, the 70 µL of plasma must be collected, diluted to 1mL total volume, and transferred into the test cartridge.

2. Background

2.1 HIV in South Africa

The latest data on HIV from the Joint United Nations Programme on HIV/AIDS (UNAIDS) reveals that globally in 2018, 37.9 million people were living with HIV, 1.7 million people were newly infected, and 770 thousand people had died of AIDS-related illnesses.[4] South Africa is home to the largest HIV epidemic in the world, where 7.7 million people were living with HIV in

[4] UNAIDS, “UNAIDS Data 2019”, [https://www.unaids.org/sites/default/files/media_asset/2019-UNAIDS-data_en.pdf], (September 20, 2019)

2018. With 20% of the global HIV population residing in South Africa, it is clear that a process needs to be developed to determine HIV viral load in order to assess a patient's progress with antiretroviral therapy (ART).

In 2014, UNAIDS launched a goal known as the 90-90-90 target, which stated that by 2020, "90% of people living with HIV will know their HIV status, 90% of people who know their HIV-positive status will be accessing treatment, and 90% of people on treatment will have suppressed viral loads."[5] Basically, the 90-90-90 target refers to 90% of all people with HIV knowing their status, 81% on treatment, and 73% with suppressed viral loads. According to the World Health Organization (WHO), HIV virological suppression is reached once a person has an HIV viral-load of less than 1000 copies/mL.[6] This goal was created in order to unite the world to end the HIV-AIDS epidemic. However, the 2018 statistics of HIV and AIDS, specifically in South Africa, indicate that these goals will not be met. In 2018 in South Africa, while 90% of people who were living with HIV knew their status, only 62% were on treatment, and just 54% had suppressed viral loads.[7]

2.2 Current HIV Diagnostics

In order to reach the 90-90-90 target set forth by UNAIDS[5], timely data regarding viral load is a necessity for patients to be diagnosed and receive ART. One way to accomplish this is point-of-care (POC) testing, which helps coordinate efforts to diagnose people living with HIV

[5] UNAIDS, "South Africa", [https://www.unaids.org/en/regionscountries/countries/southafrica], (September 30, 2019)
[6] World Health Organization, "Viral suppression for HIV treatment success and prevention of sexual transmission of HIV", [https://www.who.int/hiv/mediacentre/news/viral-supression-hiv-transmission/en/], (September 30, 2019)
[7] UNAIDS, "South Africa", [https://www.unaids.org/en/regionscountries/countries/southafrica], (September 30, 2019)

and initiate ART.[8] There are many different types of HIV tests available in the consumer market, but POC facilities in low- to middle-income countries (LMIC) have a need for different tests because of their lack of access to sophisticated laboratory infrastructure and highly skilled laboratory technicians.[9]

2.2.1 Standard Laboratory Testing

Commonly used HIV diagnostics detect HIV by looking for the associated antibodies. This is most commonly done by examining antibodies present in whole blood that has been collected through venipuncture.[10] To test for HIV, the whole blood is first assayed with an enzyme-linked immunosorbent assay (ELISA), and then diagnosis is confirmed using a more specific test, the Western Blot assay. This test requires skilled technical staff as well as a steady power supply for the equipment.[8]

Another test available is called the nucleic acid test (NAT). This test is capable of giving either a positive/negative result or the viral-load of HIV in the whole blood.[11] NAT detects levels of viral genetic material in blood and has the ability to diagnose a person with HIV at an earlier state post initial exposure than a standard blood test. However, this test is very expensive and is not routinely used to screen individuals unless they have had a high-risk exposure or have early symptoms of HIV infection.

---

[8] Drain, PK, et al., American Society for Microbiology, "Point of Care HIV Viral Load Testing: an Essential Tool for a Sustainable Global HIV/AIDS Response"[https://cmr.asm.org/content/32/3/e00097-18.full], (September 30, 2019)
[9] Manoto, S, et al., "Point of Care Diagnostics for HIV in Resource Limited Settings: An Overview, [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6037236/], (September 29, 2019)
[10] The Body, "Types of HIV Tests", [https://www.thebody.com/article/types-hiv-tests], (September 30, 2019)
[11] Centers for Disease Control and Prevention, "Types of HIV Tests", [https://wwwn.cdc.gov/hivrisk/how_know/different_tests.html], (October 8, 2019)

2.2.2 Point-of-Care Testing

Point-of-Care diagnostics in LMICs require a different set of constraints because of the different environment usually found in these countries. Many current viral load tests require sophisticated laboratory equipment that has to be operated by skilled lab technicians and involve the logistics of transporting blood samples to central clinics. Due to these logistics, it usually takes patients several weeks to months to receive their test results back, if they even receive them. In fact, 50% of patients never do.[12] POC facilities need a way to test for HIV viral load outside of the centralized laboratories, but in transitioning the testing to POC clinics, there are new restraints for the equipment and testing protocol. These constraints include the ability to:

- Remain functional in extreme environmental conditions
- Have minimal reliance on a power supply
- Include a simple process for sample collection
- Have automated equipment that allows for operation by users with minimal training

In recent years, there have been developments in POC HIV testing and there are a few options on the market. The current product that the clients use is the GeneXpert HIV-1 viral load assay by Cepheid. For this test, whole blood is collected by either venipuncture or finger stick and undergoes blood fractionation using a centrifuge. This leads to an additional 15 to 20 minutes of preparation time. After the blood has been separated, the test requires 1mL of isolated plasma be transferred into the HIV-1 viral load cartridge. The GeneXpert machine uses real-time reverse transcriptase polymerase chain reaction (RT-PCR) to quantify the viral load of

[12] Cepheid, “Cepheid Targets Development of a Point of Care HIV Viral Load Test From a Few Drops of Blood”, [https://www.cepheid.com/us/about-us/news-events/press-releases/250-cepheid-targets-development-of-a-point-of-care-hiv-viral-load-test-from-a-few-drops-of-blood], (October 8, 2019)

HIV-1 with a limit of detection of 40 copies/mL.[13] The machine takes around 90 minutes to process the results and requires a maximum temperature of 30ºC at the location of use. For WHO prequalification, the Cepheid GeneXpert HIV-1 viral load assay had "a 94% sensitivity and 99% specificity for detecting HIV viral loads of greater than 1,000 copies/mL."[14]

2.3 Existing Technologies Overview

2.3.1 Blood Collection Devices

The type of blood collection device used for a patient is determined by the amount of blood collected as well as where in the body the blood is withdrawn from. These devices come in all shapes and sizes, and use various methods for drawing blood into the instrument. According to the client, individuals from South Africa prefer a finger stick to venipuncture, and venipuncture requires more training and skill. Therefore, collection devices suited for a finger stick are needed. Two common blood collection devices for a finger stick are shown below.

[13] Nash, M, et al., "Performance of the Xpert HIV-1 Viral Load Assay: a Systematic Review and Meta-analysis", [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5869835/#B2], (October 8, 2019)
[14] Drain, PK, et al., "Point-of-Care HIV Viral Load Testing: an Essential Tool for a Sustainable Global HIV/AIDS Response", [https://cmr.asm.org/content/cmr/32/3/e00097-18.full.pdf], (October 8, 2019)

2.3.1.1 Blow-Molded Device

Figure 2: An 80 µL blow-molded capillary blood collection device

This blow-molded version of a blood collection device (*Figure 2*) is made of plastic and can hold 80 µL of blood. With the device held straight up and down so that the open end of the device (on the right side of the figure) is facing upwards and in contact with the site of the finger stick, the force of gravity enables blood to flow down the tube. A tiny hole, located near the black line, allows air to push back against the blood and stop it from flowing further down the tube after 80 µL of blood has been collected. The bulb on the other side of the black line can be squeezed to release the blood. This type of blood collection device is advantageous because of its low cost, but it has a major drawback. Since this device relies solely on gravity for blood flow down the tube, an air bubble in the tube during blood collection would result in having to start the collection process over.

2.3.1.2 Sarstedt Minivette

Figure 3: A 50 µL capillary blood collection device manufactured by Sarstedt

The Sarstedt Minivette (*Figure 3*) can hold up to 50 µL of blood. The blood is drawn into the device through capillary action, and stops flowing once it has reached the white porous frit in the middle of the device. The porous frit is made up of a collection of hydrophobic particles that are fused together. These particles repel the blood and cause it to stop flowing. Blood is expelled by pushing down on the white plunger of the device. This type of blood collection device is more expensive than the plastic blow-molded version, but users tend to like this style better because of its syringe-like feeling that gives more control.

2.3.2 Centrifuges

Centrifuges will be critical in the device design since they multiply the effects of gravity in order to expedite the separation of blood into its components, plasma, white blood cells, and red blood cells. In order to optimize the time and energy involved in the centrifugation process, the team must consider the relationship between centripetal force and parameters of the centrifuge:

$$F = \frac{mv^2}{r} = m\omega^2 r$$

Equation 1: Centripetal force for a centrifuge

where *F* is the centripetal force, *m* is the mass, *v* is the linear velocity, *r* is the radius of the rotor in the centrifuge, and *ω* is the angular velocity. Thus, as the radius of the centrifuge decreases, the angular velocity must increase in order for the sample to experience the same amount of acceleration and force.

3. Stakeholders and Users

3.1 Stakeholders

3.1.1 David Kelso, PhD

The primary stakeholder is the client, Dr. Kelso. His research and selected publications focus on ways of diagnosing and analyzing diseases in resource-poor areas. One publication describes a study in which it was found that people in South Africa prefer a finger stick over venipuncture, which is their current way of drawing blood for HIV testing. Dr. Kelso is invested in changing this collection process so that it is done through finger stick instead, with all of the testing done POC so that the patient can quickly receive their test results.

3.1.2 Patients

The outcome of this project directly affects the patients' health and quality of life. With the completion and success of the product, a patient can quickly find out whether or not they have HIV and start treating it earlier in the case that the test is positive. If the results come back

negative, they do not have to live with the stress of not knowing. This product would improve the lives of every patient, independent of what the outcome of the test is.

3.2 Users

Clinicians in South Africa are the primary users. They are the ones operating the product, so ease of use, especially, with little to no training, is important to them. The amount of time it takes to collect the blood and run the centrifuge is also an important aspect. If the clinicians must wait a while for the centrifuge to finish separating the blood into plasma and its other components, they might get bored, start working on another task, and return to the centrifuge after a longer amount of time, which is not ideal.

Some clinics use the wheel-and-spoke model for HIV testing, in which samples from local clinics are processed at a central laboratory, and this workflow works for them. However, this is not the case at clinics in more remote areas, where transportation is not as accessible. Thus, different use cases exist for these different settings.

3.2.1 Wheel-and-Spoke Clinics

These clinics should continue to send their blood samples to a central laboratory for testing, but some processing can be executed at the clinic to isolate the plasma. This plasma can be transported to the laboratory and processed further for testing.

3.2.2 Rural Clinics

Since these clinics do not have easy access to transportation, it would be ideal if they were able to execute their own testing. This would include isolating plasma, preparing it to be used with the applicable test, and running the test.

4. Collection Device

4.1 Design

The collection device design makes use of an existing product for collecting blood from a finger stick, a 200 µL Sarstedt Minivette (*Figure 4)*, and a sump that the team designed (*Figure 5)*, which the Minivette fits into and creates a seal via an O-ring. When the device is spun in the centrifuge, blood will exit the Minivette into the sump until it reaches maximum volume capacity, and air will exit the sump through the channels on the side of the capsule into the environment. The sump is designed to hold exactly 130 µL of fluid, so after centrifugation, 130 µL will be collected in the capsule while 70 µL remains in the Minivette. Because the densest components will go towards the bottom first, the 70 µL that remains in the Minivette will be the plasma that will be used for further testing with the GeneXpert, while the 130 µL in the sump will consist of red blood cells, white blood cells, and excess plasma.

Figure 4: 200µL Sarstedt Minivette.

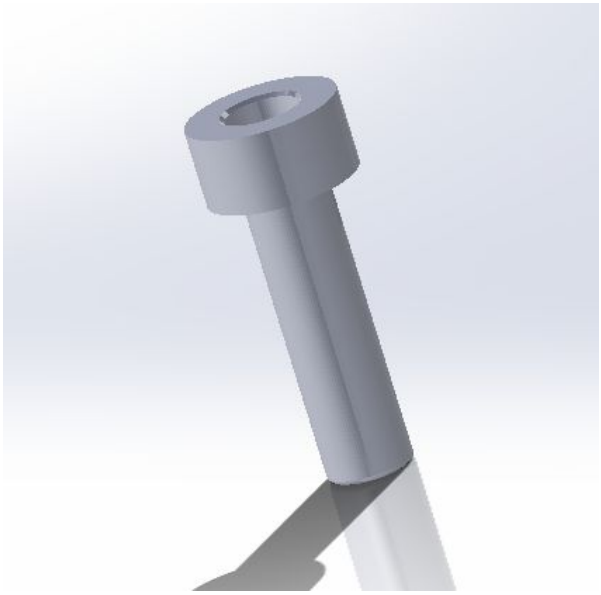


Figure 5: 130 µL sump with hidden O-ring.

4.2 Testing

4.2.1 Initial Blood Separation Testing

The initial testing with porcine blood consisted of two parts. FIrst, a 100 µL Sarstedt Minivette blood collection device was tested to determine if the Minivette could even emit blood during centrifugation. This was done by filling the Minivette with 100 µL of porcine blood. Two devices were placed across from each other in a Mini Mouse II centrifuge in order to keep the weight balanced (*Figure 6*). After a seven-minute centrifugation, the Minivette was taken out of the centrifuge. The device had a majority of the blood collected in the capsule (*Figure 7*), showing proof-of-concept that it is feasible for the design to rely on centripetal force to expel the unwanted erythrocytes, leukocytes, and extra plasma.

Figure 6: Setup of two prototypes in the Mini Mouse II centrifuge.

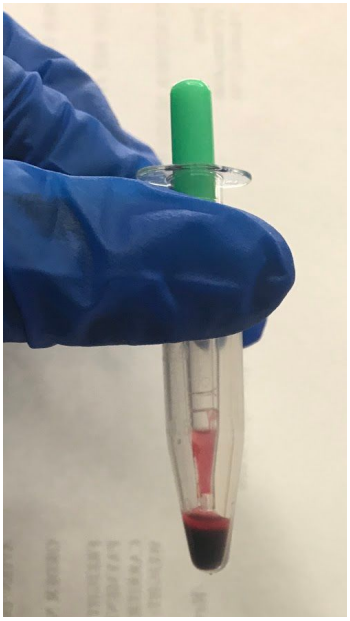
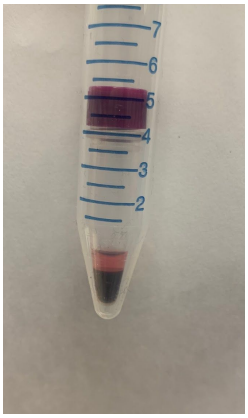
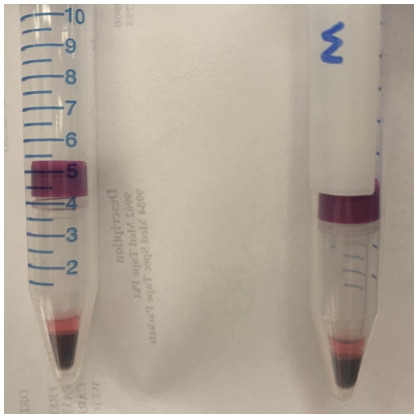


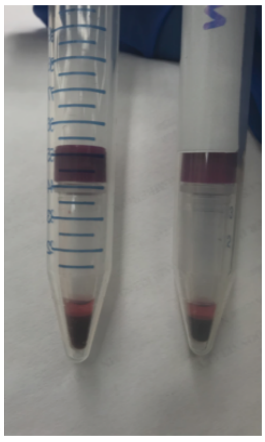
Figure 7: Results of spinning prototype for seven minutes.

The next part of the testing used the Mini Mouse centrifuge as well as the Eppendorf centrifuge. For these tests, microfuge tubes were filled with 200 µL of porcine blood. These tubes were put into the centrifuges and run at known rpms in order to determine the times and

angular velocities needed to sufficiently separate blood. The angular velocity can be adjusted on the Eppendorf, so separation tests were run at 4400 rpm, 2200 rpm, and 1100 rpm. The minimum amounts of time to effectively achieve plasma separation were three minutes, four-and-a-half minutes, and seven-and-a-half minutes, respectively (*Figures 8a-c*). The minimum time for separation was tested on the Mini Mouse II as well since the design of this centrifuge is closer in geometry to the team's centrifuge design concepts. The Mini Mouse II runs at 6000 rpm, and the minimum time was found to be two-and-a-half minutes (*Figure 8d*).

a) 

b) 

c) 

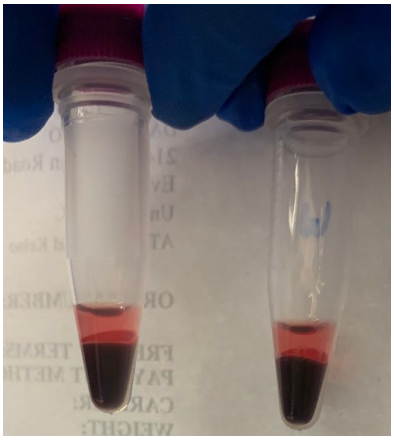
d) 

Figure 8: a) 200 µL of blood spun at 4400 rpm for 3 minutes. b) 200 µL of blood spun at 2200 rpm for 4.5 minutes (left) compared to control (right). c) 200 µL of blood spun at 1100 rpm for 7.5 minutes (left) compared to control (right). d) 200 µL of blood spun in Mini Mouse II at 6000 rpm for 2.5 minutes (left) compared to control (right).

4.2.2 Prototype Testing

The team conducted initial testing with the prototype using the Eppendorf centrifuge in the CIGHT lab. Dyed water was first used for testing in order to observe what the fluid is doing during the process *(Figure 9*). An unexpected problem was that fluid leaked out of the Minivette even before centrifugation, which is suspected to be due to gravity and pressure from the blood and the lack of capillary action. However, this may not be a significant problem, as the blood is still able to separate into components.

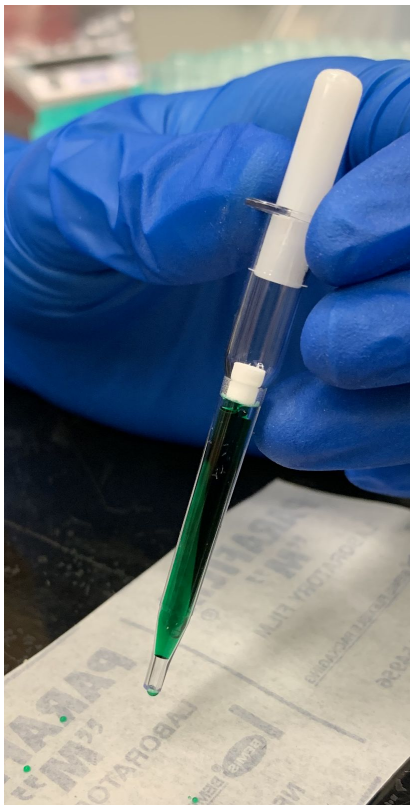


Figure 9: Sarstedt Minivette with green water for testing.

Looking at the fluid balance, the plunger of the Sarstedt seemed to be pushing more of the fluid out than desired, as the heights appeared to be unequal, so the team decided to run the centrifuge without the plunger. When tested with porcine blood, the heights visually

appeared to be equal (*Figure 10*), but there only about 25µL was left in the Minivette, so the team decided in future development to reevaluate the geometry of the Minivette and capsule while the device is horizontal in the centrifuge.

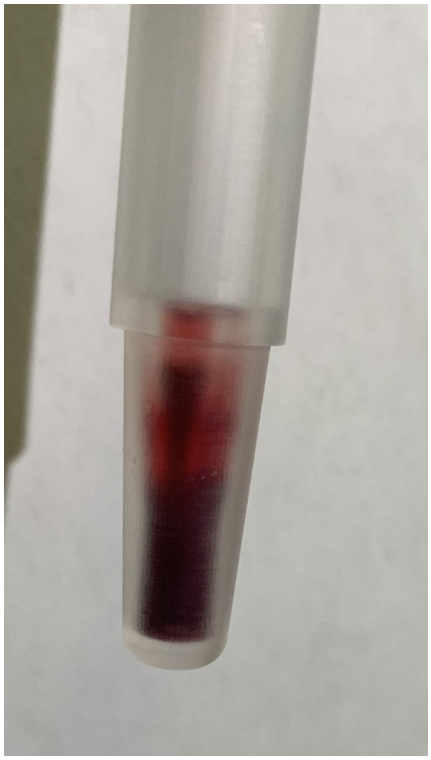


Figure 10: Result of centrifugation without Sarstedt Minivette plunger.

4.2.3 200 µL Sarstedt Minivette Testing

Although the Sarstedt Minivette claims that it holds 200 µL of blood, it was important for the team to verify this. This is of high importance because if the Sarstedt does not, in fact, hold 200 µL of blood every time a user attempts to draw blood, the different amounts of whole blood may affect the amount of plasma collected and the overall balance of the centrifuge.

To test the amount of blood the Sarstedt actually held as well as whether different users were able to draw the same amount of blood, three subjects attempt to fill the Sarstedts using

porcine blood and parafilm. Each user was asked to fill 12 Sarstedts completely using gravity to guide the blood into the opening of the Sarstedt. After each trial, the Sarstedt was weighed on a tared scale to determine the amount of blood in the Sarstedt. This data can be seen in the figure below.

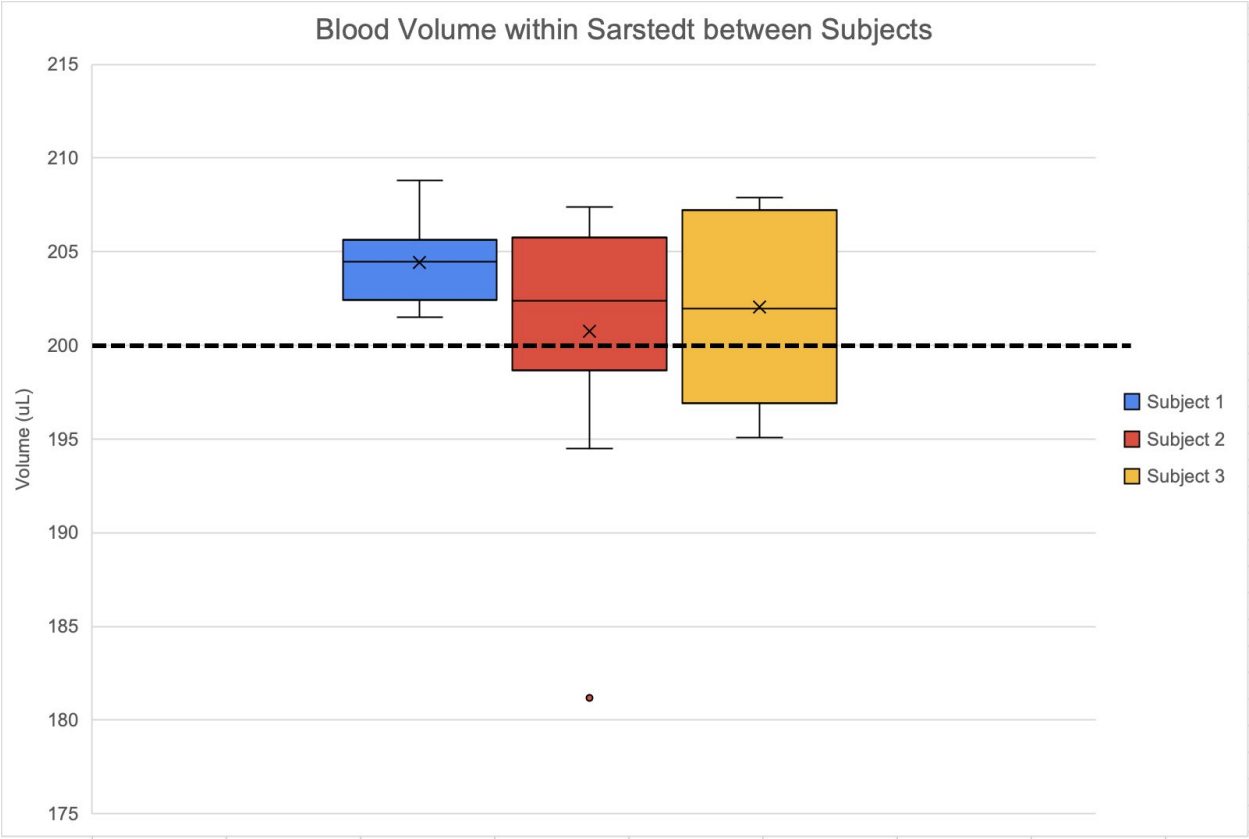


Figure 11: Blood Volume within Sarstedt Between Subjects

As seen in Figure 11, all subjects were able to collect 200 ± 5 µL of blood, with one outlier performed by Subject 2. In a future section of this report, it will be proved that even with ± 5 µL of collected blood, the required 70 µL of blood was still obtained. However, this needs to be analyzed further to see if it will affect the balance of our centrifuge. Furthermore, when an ANOVA test was performed, the p-value equaled 0.23 which means that there is not sufficient evidence to say that the subjects are collecting different amounts of blood. However, post-hoc analysis with our current testing procedure only gave this test a power of 0.3. Therefore, in the

future, our group would like to rerun this test with more subjects and more trials. According to an A-Priori test, to achieve a power of 0.8 with 10 subjects, each subject needs to perform this test 19 times.

4.2.4 Sump Testing

In order to test the sump, the team collected 200 µL of porcine blood into the 200 µL Sarstedt Minivette. The Minivette was then placed into the sump and this entire unit was then centrifuged at different times and speeds. To collect the data, the team started by weighing the Sarstedt, on a scale tared to the Sarstedt, after the porcine blood was collected -- this gave the initial amount of blood in the Sarstedt. The team also weighed the sump prior to each trial in order to get a baseline value. After centrifuging, the sump was re-weighed meaning that the blood left in the sump is just the difference between the weight of the sump after centrifugation and before. Lastly, the team was able to calculate the fluid remaining in the Sarstedt by finding the difference between the initial weight of the blood in the Sarstedt and the weight of the blood in the sump. There are two shortcomings in this experiment design. The first is that the team assumed no fluid was lost throughout the entire process. The team also assumed that the density of blood is 1 and therefore 1 mg corresponds to 1 µL. The data collected is illustrated in the table below.

Table 1: Data from Sump Collection Testing

RPM	Time (min)	Trial	Weight of Blood in Sarstedt (mg)	Weight Cap Before (mg)	Weight Cap After (mg)	Blood Left in Cap	Blood Left in Sarstedt	Appearance of Blood in Sarstedt
4400	7.5	1 (B)		2072.7	2199.8	127.1	72.9	Clear red
		2 (A)		2058	2187.5	129.5	70.5	Clear red
		3 (A)	207.3	2059.4	2186.3	126.9	80.4	Clear red

		4 (B)	200.7	2073.8	2201.5	127.7	73	Clear red
	10	5 (A)	206.9	2059.8	2184.4	124.6	82.3	Clear red
		6 (B)	207.9	2074.6	2199.6	125	82.9	Clear red
		7 (A)	207.2	2060	2183	123	84.2	Clear red
		8 (B)	197	2074.5	2199.8	125.3	71.7	Clear red
	15	9 (A)	195.1	2060.5	2184.2	123.7	71.4	Clear red
		10 (B)	198.5	2075	2199.9	124.9	73.6	Clear red
		11 (A)	203.2	2060.8	2187	126.2	77	Clear red
		12 (B)	196.6	2075	2199.3	124.3	72.3	Clear red
2200	7.5	13 (A)	197.9	2058.5	2186.7	128.2	69.7	Clear red top half, dark red bottom half
		14 (B)	181.2	2073.3	2201.1	127.8	53.4	Clear red top half, dark red bottom half
		16 (A)	206.4	2058.6	2184.8	126.2	80.2	Clear red
		17 (B)	201.9	2075.8	2200.3	124.5	77.4	Clear red
	10	18 (A)	203	2060.7	2184.6	123.9	79.1	Clear red
		19 (B)	201	2075.2	2199.6	124.4	76.6	Clear red
		20 (A)	202.2	2060.6	2184.7	124.1	78.1	Clear red
		21 (B)	207.4	2075.2	2210.4	135.2	72.2	Clear red
	15	22 (A)	194.5	2060.7	2185.5	124.8	69.7	Clear red
		23 (B)	206.9	2075.2	2199	123.8	83.1	Clear red
		24 (A)	202.6	2060.9	2185.7	124.8	77.8	Clear red
		25 (B)	203.9	2075.5	2199.8	124.3	79.6	Clear red
4400	4	1 (B)	201.5	2075.5	2201.9	126.4	73.6	bright red
		2 (A)	206.9	2058.4	2186	127.6	72.4	bright red
		3 (A)	203.7	2060	2187.2	127.2	72.8	bright red
		4 (B)	205.4	2075.2	2202	126.8	73.2	bright red
	7.5	5 (A)	205	2061.2	2189.2	128	72	bright red
		6 (B)	204.4	2077.2	2217.6	140.4	59.6	bright red
		7 (A)	204.5	2061.5	2186.5	125	75	bright red
		8 (B)	205.7	2077.8	2203.7	125.9	74.1	bright red
	15	9 (A)	208.8	2061.7	2188.5	126.8	73.2	bright red
		10 (B)	202.8	2078	2203.6	125.6	74.4	bright red

		11 (A)	202.1	2061.9	2190.8	128.9	71.1	bright red
		12 (B)	202.3	2078.6	2206.4	127.8	72.2	bright red

From this data, which is more clearly illustrated in the following figures, it is shown that at 4400 RPM, no matter how much whole blood was collected initially, the sump still collected the required 70 µL of plasma for each trial except one trial where the O-ring did not hold its seal. However, the plasma obtained in each trial has not been tested yet to see if the required 95% of leukocytes were removed, this is something that the team will perform in future testing. As seen in figure _, at 2200 RPM, the sump did not always successfully collect 70 µL of plasma.

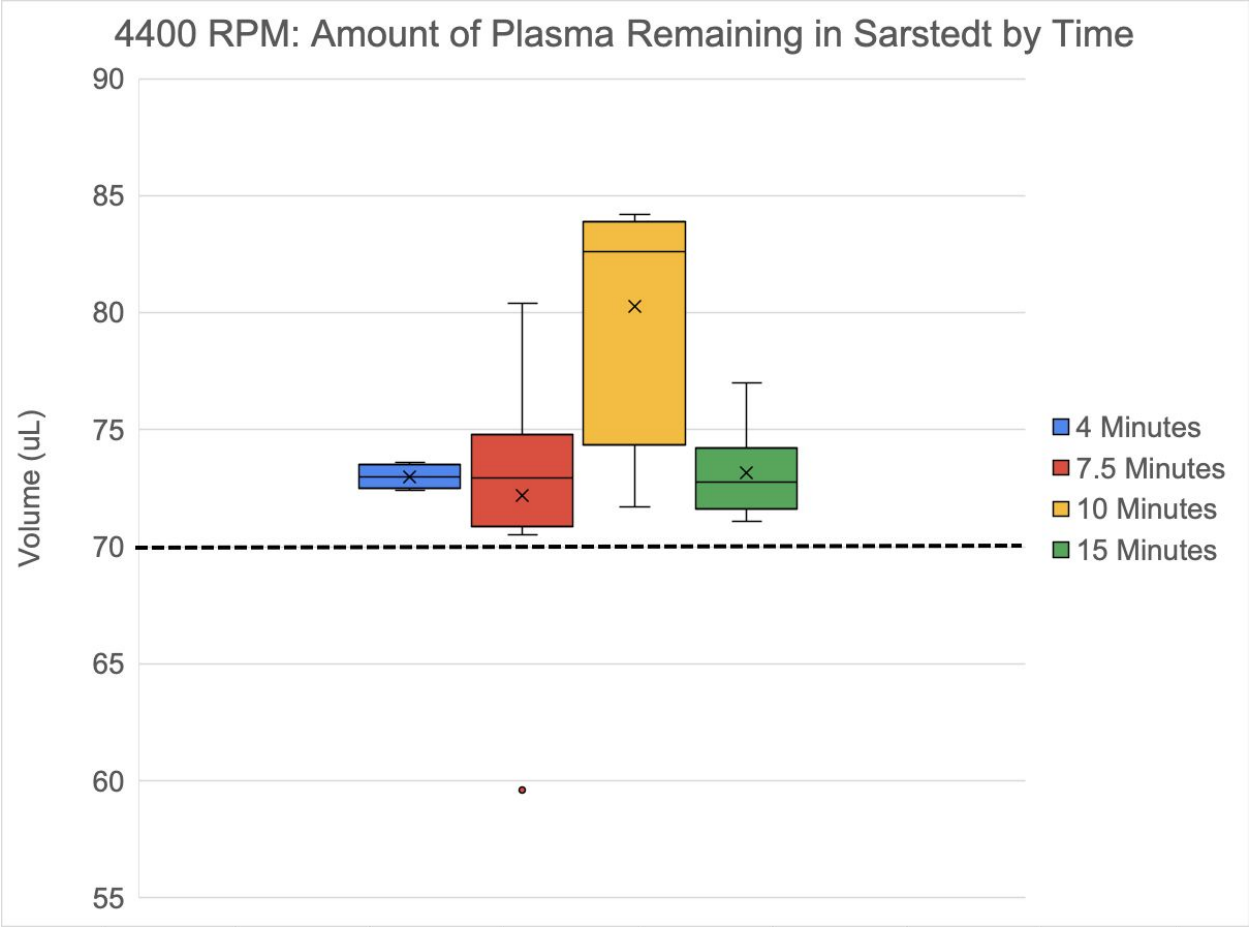


Figure 12: Amount of Plasma Collected at 4400 RPM

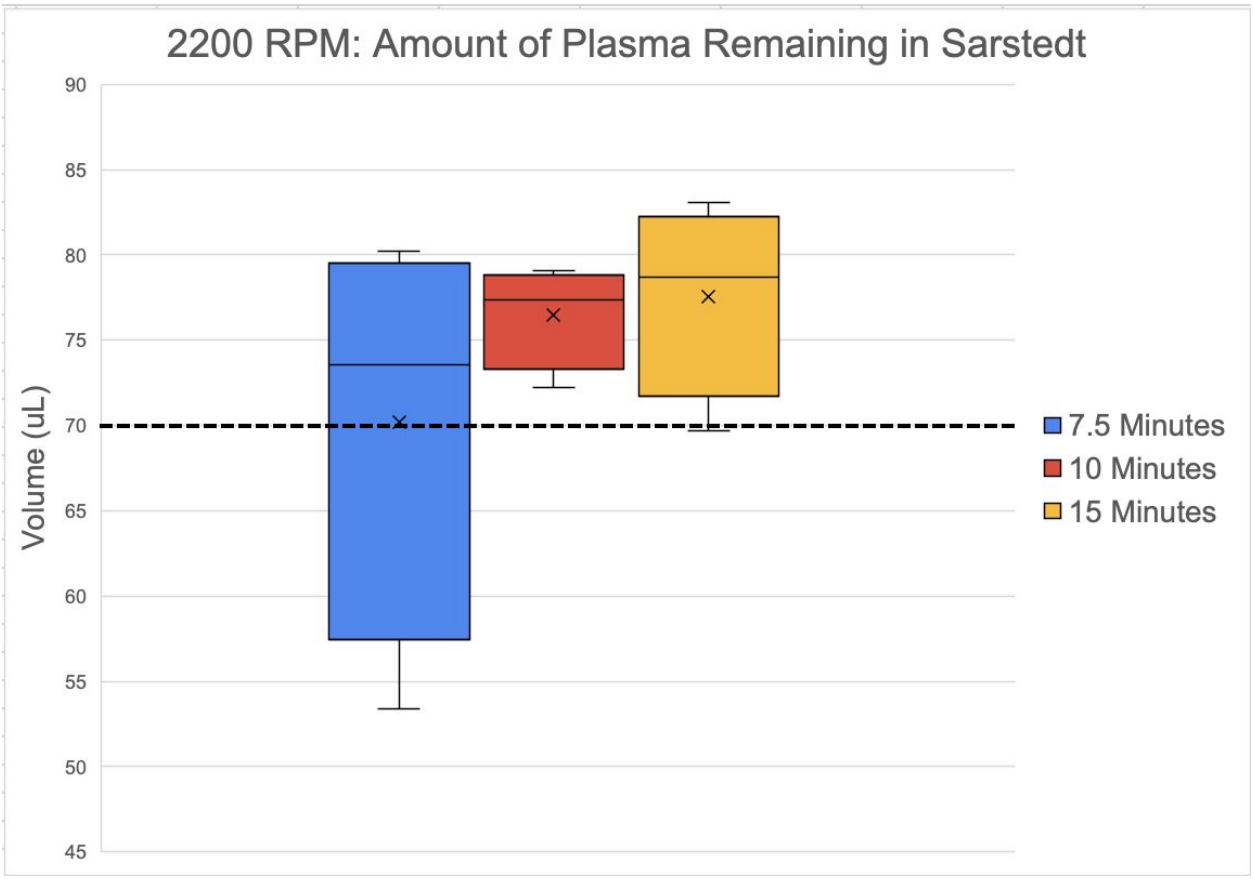


Figure 13: Amount of Plasma Collected at 2200 RPM

Although the collected plasma has not yet been tested to see whether 95% of leukocytes have been removed, the plasma was visually assessed in order to see the difference between the plasma and remaining mixture of leukocytes, erythrocytes, and extra plasma. This can be seen in the figure 14 below, where the clear, bright red fluid in the Sarstedt is the plasma and the darker, maroon fluid is the mixture. To note, the plasma is not clear because the porcine blood is hemolyzed.

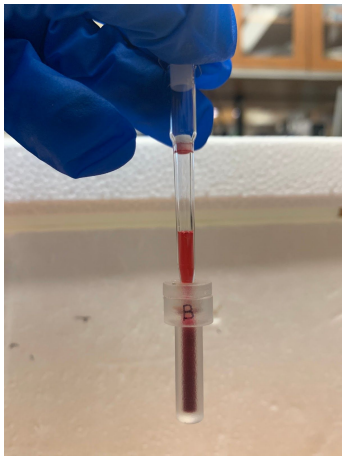
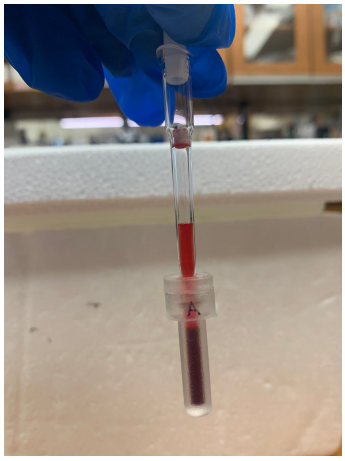
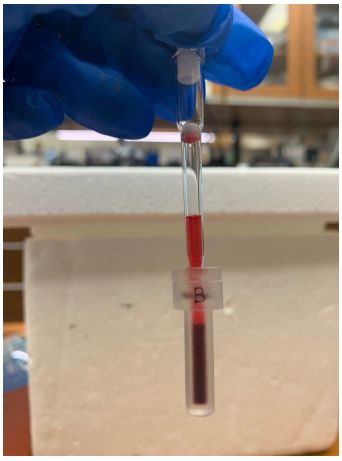
a)  b)  c) 

Figure 14: a) Sample collected at 4400 RPM spun for 4 minutes. b) Sample collected at 4400 RPM spun for 7.5 minutes. c) Sample collected at 4400 RPM spun for 15 minutes

Once the team is able to test the plasma for leukocyte removal, this data will then be re-examined in order to pick the ideal speed and time to run the sample for. However, more testing may be needed in the future.

5. Centrifuge

5.1 Design

The 3-d printed and assembled centrifuge is shown below in Figure 15(a). This design is slightly different than the CAD model shown in (b) and (c), because it has recently been updated in response to feedback from user testing in South Africa, which is discussed later. The way the centrifuge works, however, is the same.

The centrifuge consists of a 12 V motor and a circular rotor. The rotor has a slot for the device with ribbing inside to keep the device in place while it is being centrifuged as well as a hinged door to prevent the device from flying out. As seen in Figure 15(b), there is a wider slot

opening at the left end of the rotor that allows the user to insert and remove the device more easily. Opposite the wider opening, there is a cutout that places the center of mass at the center of the rotor. The placement of the device puts the plunger of the Minivette on the opposite side of the center of the rotor as the rest of the Minivette, so that the centripetal force will drive the plunger out of the Minivette. However, it is stopped by the wall of the slot, and the plunger therefore will not affect the amount of liquid in the Minivette. The centrifuge is encased in a white box that closes in order to make sure nothing flies out during centrifugation.

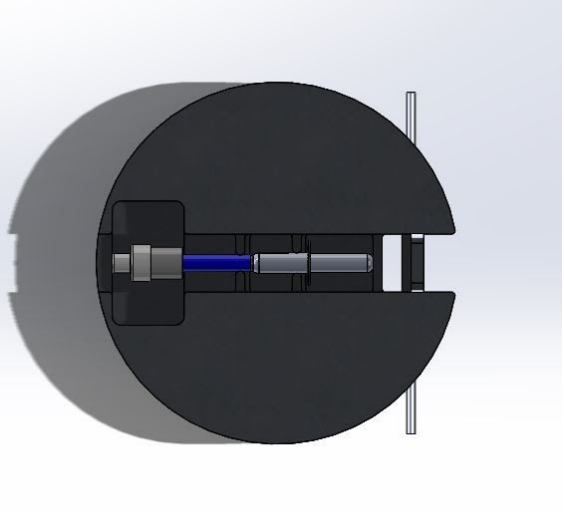


Figure 15: Centrifuge design a) old design in box b) with device c) side view.

5.2 Testing

5.2.1 Motor RPM Calculations

The table below, table 2, lists out various angular speeds specific to a certain G-Force. These calculations were based off of the required radius of our rotor, 95 mm, and the mass of 200 µL of whole blood, 0.0002 kg. These values were then used in coordination with the centrifuge testing at various rpm, in order to distinguish which motor is needed for the centrifuge.

Table 2: RPM calculations for a given G-Force

Calculations			
Amount of G's	**Centripetal Force**	**Angular Speed (rad/s)**	**Rpm**
1000	1.962	321.345854	3068.626867
1500	2.943	393.5666867	3758.285017
2000	3.924	454.451665	4339.693733
2500	4.905	508.0924077	4851.925094
3000	5.886	556.587346	5315.017642
3500	6.867	601.1830442	5740.875191
4000	7.848	642.691708	6137.253733
4500	8.829	681.6774974	6509.540599
5000	9.81	718.5511739	6861.658271
5500	10.791	753.622829	7196.567908
6000	11.772	787.1333733	7516.570034

5.2.2 Centrifuge Testing

While the team intended to conduct some initial centrifuge testing, they were only able to set up the current prototype because certain components did not arrive in time and because

there were many design iterations of the sump that led to changes in the centrifuge prototype as well. However, the team was able to obtain feedback on the current prototype from members who performed initial user observations in South Africa, this will be discussed in further in the report. With these observations, the team was able to update their current centrifuge to prepare it for testing in the future.

Furthermore, the concepts and questions to investigate once initial testing begins have been determined. While the chosen motor claims to spin up to speeds of 7420 rpm, with the added weight of our rotor, this may not be the case, so the first thing to test will be the actual speed of the centrifuge. In addition to the speed, the security and integrity of each component will need to be tested, both to make sure that the collection device will be stable within the rotor and to make sure that the rotor does not bow and fall apart. Balance of the rotor was checked through CAD modelling, but needs to be checked again after complete assembly and during the centrifugation process. Finally, the team will need to determine how long and at what speed a blood sample needs to be spun for to induce the plasma separation to remove 95% of leukocytes for this centrifuge geometry.

6. User Observation and Expert Interviews

While three members of the team were performing quantitative testing and updating the design concepts, there were two members in South Africa who were able to perform user testing as well as conduct expert interviews with stakeholders at POC clinics. Through these expert interviews and user observations, the team based in Evanston was able to update current designs and send the updates back over to South Africa to go through another round of user-testing.

6.1 Expert Interviews

6.1.2 Grant Theron

Professor Grant Theron is the principal investigator of the Clinical Mycobacteriology and Epidemiology (CLIME) Group at Tygerberg Hospital, a tertiary hospital in Western Cape, South Africa. His studies focus on TB diagnostics, specifically the disease's transmission and pathogenesis. He recommended that the prototype built is used as a POC device for primary and secondary hospitals. He emphasized the need for minimal training, one of the largest hurdles for efficient implementation of POC technologies. He suggested that both research and community nurses will be able to give helpful feedback in user observation and testing of the prototype. Professor Theron sees this prototype as a promising addition to the current South African wheel-and-spoke model.

6.1.2 CLIME Laboratory Observation

The focus of the CLIME Group is to improve how patients with tuberculosis (TB) or drug-resistant TB are diagnosed, enhance the understanding of TB transmission, and investigate whether the microbiome has any role in TB pathogenesis. During the visit to their lab, the team observed two processes for TB diagnosis: urine sample processing and blood sample processing.

The goal of urine sample processing at the CLIME lab is to diagnose and treat TB patients before the symptoms start to appear. Some patients also cannot produce sputum, so a urine test may be an optimal alternative to a sputum test. The urine sample processing steps are:

1. Separate and prepare samples by patient (2 samples/patient)
2. Choose the more concentrated sample of urine to work with and freeze the other for biobank
3. Centrifuge for 10 minutes
4. Remove all but 750 µL of the supernatant
5. Vortex and mix the pellet into the remaining 750 µL supernatant
6. Add 1.5 mL of buffer
7. Vortex for 1 minute
8. Incubate for 10 minutes
9. Vortex for 1 minute
10. Incubate for 5 minutes
11. Load the sample into a GeneXpert cartridge

The validity and reliability of urine tests have yet to be confirmed, but successful correlation between urine and sputum sample tests can lead to benefits of an alternative TB diagnostic test.

Figure 16: Blood sample processing in the CLIME lab.

The CLIME lab also receives blood samples from patients with TB from three different local clinics, which are processed and biobanked for collaborators' future studies by the researchers (*Figure 5*). The steps of blood sample processing are:

1. Prepare labels with appropriate sample info for microfuge tubes ahead of time based on info sent from nurses
2. Balance samples (except those collected in sodium citrate) to be centrifuged. For the most part, patients have 5 samples each - 2 without an anticoagulant, 1 in heparin, 1 in EDTA, and 1 in sodium citrate
3. Centrifuge for 10 minutes
4. Organize samples in the test tube rack such that samples are grouped by patient.
5. Prepare enough preservative solution for all samples of buffy coat, consisting of 1:9 DMSO:FBS
6. Prepare samples for one patient at a time
    a. Open all the caps for one patient's microfuge tubes
    b. From samples without anticoagulant, collect 3 500µL samples of serum (supernatant)
    c. From samples in heparin and EDTA, collect 2 500µL samples of plasma (supernatant) and 1 sample of buffy coat. To collect a buffy coat, use a pasteur pipette to pipette off remaining plasma, and then pipette up buffy coat while collecting as little of the red cells as possible. Add 500µL preservative to buffy coat samples and mix
    d. Close tubes as each vacutainer sample is completed
    e. Pour liquid waste into 10% bleach and place solid waste in biohazard bag throughout

7. Biobank samples at -80°C
8. Record information in an Excel spreadsheet

According to the researcher performing these tasks, it is frustrating when there is not enough blood to produce the necessary samples. The fast plasma separator device should take in a consistent amount of whole blood and produce a consistent amount of plasma.

6.1.3 Benjamin Botha

Dr. Benjamin Botha is a clinical manager at the Western Cape Department of Health. His responsibilities include seeing patients, finding medications, and monitoring lab tests. He emphasized that POC testing should only be implemented when it adds value for the patient and for the health care system at the point of care. This is often not the case with HIV viral load testing in South Africa, though a niche market could be testing for HIV in newborn babies. However, a cheap centrifuge could be very valuable in many areas, including testing for potassium levels, testing bilirubin levels in infants, and producing platelet-rich plasma.

6.1.4 Andrew Whitelaw & Wolfgang Preiser

Professor Andrew Whitelaw is an associate professor and a principal specialist at the Division of Medical Microbiology of University of Stellenbosch. Professor Wolfgang Preiser is a pathologist and a consultant virologist at the Division of Medical Virology of University of Stellenbosch. Both experts gave helpful critiques, pinpointing potential concerns regarding the integration of the prototype into the current South African healthcare system. The feedback is summarized below:

1. Staff training
    a. Who trains them? Who gets trained?

    b. How is the training managed? How is quality training ensured?
    c. What if trained staff goes on a leave? Holidays?
2. Anti-coagulants: How long can the blood sample be left out before centrifuging? What is the minimum or maximum incubation period? Would the samples be stable after?
3. Identification: Will there be stickers or barcodes with patient information on the samples?
4. Bottleneck in procedure: Can more than one sample run at the same time?
5. Threshold: 1000 copies/mL of HIV viral-load defined by WHO may be too high.
6. Risks
    a. What if less than 200 µL is drawn by the Minivette?
    b. Who dilutes the 50 µL plasma sample with PBS before putting the sample into GeneXpert cartridge? How can it be ensured that the dilutions are done correctly? Who does the dilutions?
7. Financial feasibility: Not many hospitals and clinics have GeneXpert machines onsite.
8. Mixed model
    a. Current wheel-and-spoke model works for some hospitals, as it is cheaper to have samples taken by a truck than to implement a new POC testing procedure.
    b. Remote locations struggle with the wheel-and-spoke model, so POC technologies may be the solution.
9. Design - device
    a. As few moving parts as possible
    b. Something to stop users from pushing in the Minivette plunger
    c. Preassembled cap will promote simplicity
    d. More closed the system, the better
10. Design - centrifuge

a. Automated runtime of the centrifuge for 10 minutes
b. Visual indicator that indicates centrifuge speed and alerts malfunction
c. Counting the number of samples centrifuge processed will help users know when to recalibrate or to order a new device

11. African Society of Laboratory Medicine (ASLM)
    a. Look into who's using what platform where
    b. Look into what other countries are using
12. Ongoing debate: Should nurses be allowed to perform POC testing?

The critiques and feedback from the expert interviews helped shine light on the current design and its flaws in the context of the South African healthcare system. These flaws and limitations were discussed in detail with the Evanston team in order to implement appropriate modifications to the prototype design.

6.2 Clinic Visits

The team visited the caravans used for TB and HIV patients at the Kraaifontein, Wallacedene, and Scottsdene Community Health Centers (*Figure 17*) in order to observe the processes there and to conduct user testing of the design. Currently, only Kraaifontein uses POC testing.

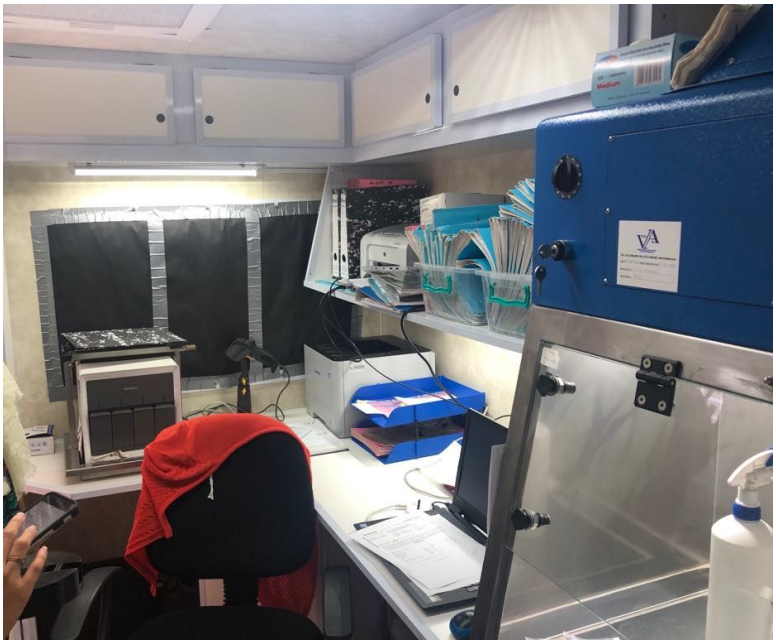


Figure 17: Caravan space used for TB and HIV patients in clinics.

To gain more information about the current process for HIV viral load testing, the team asked the nurses the following questions:

- What does HIV testing currently look like?
- How long does it take to draw blood for HIV testing?
- Do you have any problems with the current HIV testing process?
- What do you like and dislike about the current HIV test?
- How long does it take to receive patients' HIV results from laboratories?
- Do any patients not receive treatment due to problems with the testing process?
- How many patients do you see a day? How many of those are new patients waiting for diagnosis?

With only the information that the device was for POC HIV testing, two nurses were asked to assemble the device and centrifuge. One nurse was able to fit the capsule on the end of the Minivette, while the other struggled more. Both nurses struggled with putting the device in

the centrifuge due to obstruction by the door hinge and lack of clarity regarding the Minivette's orientation in the slot. The team also asked the nurses the following questions to get their opinions on the design:

- What is the main difficulty when working with this device?
- What problems do you foresee when working with this device?
- What is your favorite / least favorite part about this device?
- Do you think the finalized version of this device could have a place in this clinic?

The door hinge was updated in the design as a result, and the orientation can be specified by simple indicators on the centrifuge, similar to battery symbols indicating positive and negative. Another possible way to specify the orientation of the Minivette is by using foam with a cut-out of the silhouette of the device in the rotor. These recommendations will be looked into further in future design iterations.

7. Current Limitations and Future Developments

7.1 Current Limitations

After prototype development and more testing, the team found the following limitations with the device as well as the testing process:

- Team used hemolyzed blood, resulting in lack of plasma purity
- Team was not able to perform a finger prick, resulting in unrealistic blood collection
- Collection device has not been tested in finalized centrifuge prototype

The team plans on addressing each of these limitations in future developments.

7.2 Future Developments

7.2.1 User Observations

After initial user observations in South Africa, the team made design iterations to both the capsule as well as the centrifuge to better fit user needs. More user observations with the finalized design iterations must be conducted with the nurse practitioners, in order to determine if the changes implemented in the designs were successful, and just to gather more feedback.

7.2.2 Centrifuge Testing

The team must conduct more accurate performance testing to verify function of the devices. Looking specifically at the centrifuge, the team must make sure that the centrifuge and respecting box are machined into compatible and feasible shapes. This is to prevent aerodynamic issues that may accidentally create a “siren”, in which air gets locked into various crevices. Testing includes determining the optimal time for centrifugation at a set rpm, and looking into ways to allow the centrifuge to reach the designated speed by a set time and spin for a specific duration consistently.

7.2.3 Plasma Testing

Plasma testing consists of testing the purity of the plasma obtained post-centrifugation for each time limit. The team communicated with the client and found the most viable method to be flow cytometry, which is used to detect and count cells of certain characteristics by first suspending a sample containing cells into a fluid and injecting the sample into a flow cytometer

instrument. CD14 and CD33 are the most common cell surface markers used for the identification of leukocytes, tha can be stained and then detected using this process.

7.2.4 Cost Analysis

While the initial prototypes of the blood collection device and centrifuge were created using 3D-printing and less costly materials, the final devices will be made of different components and materials, as they must be able to withstand multiple uses and the environment in which they are used. For this reason, the team must conduct a cost analysis to determine how affordable these devices would be in public hospitals and clinics in South Africa.

7.2.5 Materials Testing

The initial prototypes of both the blood collection device and the centrifuge were fabricated using materials that were chosen because of their ease to work with. However, it is important to look into other materials that are suitable for the respective requirements. The team plans on performing additional research in this area in order to choose optimal materials for both the capsule as well as the centrifuge.

7.2.6 Risk Analysis: Safety and Usability Features

The team uncovered many limitations of the centrifuge when performing risk analysis, as shown in table 3. These limitations include the possibility of the centrifuge running without a sample placed inside or having the cover completely shut. Errors like these can be mitigated by adding an accelerometer to the centrifuge and a kill switch that prevents the centrifuge from running unless balanced correctly. These features will be developed and implemented into future design.

Table 3: Failure Mode and Effects Analysis

1	2	3	4	5	6	7	8	9	10
Function	**Failure Mode**	**Effect(s) of Failure**	**Mechanisms(s) / Cause(s) of Failure**	**Current Controls**	**S**	**O**	**D**	**RPN**	**Recommended Corrective Actions**
Blood collection	NP collects too little blood	Might not obtain enough plasma	User error, no indication on sarstedt	Wide range of acceptable amount	2	4	3	24	NP training, more secure porous frit in Sarstedt
	NP collects too much blood	Blood may not separate properly	User error, no indication on sarstedt	Wide range of acceptable amount	2	4	3	24	NP training, more secure porous frit in Sarstedt
	Blood has bubbles	Might not obtain enough plasma	User error, no indication on sarstedt	None	2	4	2	16	NP training, redesign of Sarstedt
Cap attachment	Cap is too loose	Centrifuge may be un-balanced, cap could fall off which would leak com-ponents	No indication or feedback mechanism on cap	None	3	4	4	48	Should not fit in centrifuge unless cap is on to certain level, test with range
	Cap is too tight	Centrifuge may be un-balanced, cannot dis-connect Sarstedt properly at end of process	No indication or feedback mechanism on cap	None	3	4	4	48	Cap should not be able to be tightened past certain limit
Placement into centrifuge	NP places Sarstedt in wrong orien-tation	Centrifuge may be un-balanced, ineffective blood separation	No directions for proper placement, lack of sensor	Only fits in centrifuge in 1 orientation	2	1	1	2	Add additional ribs to guide NP, add picture of Sarstedt to bottom of pocket

1	2	3	4	5	6	7	8	9	10
Function	**Failure Mode**	**Effect(s) of Failure**	**Mechanisms(s) /Cause(s) of Failure**	**Current Controls**	**S**	**O**	**D**	**RPN**	**Recommended Corrective Actions**
Placement into centrifuge	NP places Sarstedt in without the cap	Centrifuge may be un-balanced, Sarstedt would leak every-where and we would lose all plasma, contami-nation	No directions for proper placement, lack of sensor	None	4	2	4	32	Add sensor to ensure that centrifuge does not start unless balanced
	Plunger of Sarstedt is not in correct position	Leakage of collected sample before or during centri-fugation	No directions for proper placement, lack of sensor	None	1	4	4	16	Double check center of mass, add latch to lock plunger in place
	NP forgets to place Sarstedt	Centrifuge may be un-balanced, and no result for process	Lack of sensor	None	3	2	4	24	Add sensor to ensure that centrifuge does not start unless balanced
	Sarstedt bounces around during centri-fugation	Sarstedt may leak early or cause the centrifuge to become un-balanced	Compartment for Sarstedt is not secure to device	Ribs within centrifuge to hold Sarstedt in place	2	2	2	8	Add more ribs to centrifuge design
Closing lid	Door does not securely shut	Sarstedt may fly out, causing centrifuge unbalance	Design flaw (not enough space to shut due to improper measurements of door/notch)	Relying on force generated by centrifuge	3	2	1	6	Adding a latch or automatic ‘lock’ (such as with a magnet) to keep the door shut

1	2	3	4	5	6	7	8	9	10
Function	**Failure Mode**	**Effect(s) of Failure**	**Mechanisms(s) / Cause(s) of Failure**	**Current Controls**	**S**	**O**	**D**	**RPN**	**Recommended Corrective Actions**
Closing lid	Door does not open wide enough to put sample into centri-fuge	Cannot begin process of loading the sample	Design flaw	Design of centrifuge allows for enough opening	2	1	1	2	Double check design
	Door does not stay shut	Sarstedt may fly out, causing centrifuge unbalance	Design flaw (no secure attachment of door once closed)	Relying on force generated by centrifuge	3	2	1	6	Adding a latch or automatic 'lock' (such as with a magnet) to keep the door shut
Centri-fugation	Centri-fuge is too slow	May take longer time to separate blood	Motor power is not enough, size and mass of rotor is too large for motor	Relying on previous calcu-lations	1	3	2	6	Reexamine calculations, do additional testing with motor and rotor designs
	Centri-fuge is too fast	Could cause damage to rotor and motor, forces may be too strong causing parts to break	Motor power is too much, incorrect force calculations	Relying on previous calcu-lations	4	3	3	36	Reexamine calculations, redo testing, implement sensor to prevent motor from going past certain speed
	Motor shaft cannot with-stand force	Motor break causes damage to centrifuge, cannot separate blood	Improper pressure, stress, or inertia calculations, did not pick an appropriate motor	None	5	3	4	60	Perform testing on motor shaft, implement maximum speed for motor so force generation cannot exceed this limit

1	2	3	4	5	6	7	8	9	10
Function	**Failure Mode**	**Effect(s) of Failure**	**Mechanisms(s) / Cause(s) of Failure**	**Current Controls**	**S**	**O**	**D**	**RPN**	**Recommended Corrective Actions**
Centri-fugation	Rotor is not properly fast-ened to motor shaft	Rotor breaks off, process cannot be per-formed	Improper connection between two parts	None	5	2	4	40	Redo bearings
	Rotor cannot with-stand force	Rotor breaks, cannot separate blood	Improper pressure, stress, or inertia calculations, did not pick an appropriate motor	None	4	4	4	64	Perform testing on motor shaft, implement maximum speed for motor so force generation cannot exceed this limit
	Centri-fuge becomes un-balanced	Hazard/ damage caused to rotor or motor	Balance miscalculations, security failures	None	4	4	4	64	Add sensor to ensure that centrifuge does not start unless balanced
	Centri-fuge takes too long to reach proper speed	May take longer time to separate blood	Motor power	None	2	3	3	18	Research and purchase a more efficient motor
Plasma isolation	Plasma sepa-rated properly	Not pure enough to detect viral load	Design flaw with compartments, not separated for long enough	None	4	2	4	32	Reexamine designs, run centrifuge for longer time
	Not enough plasma remains in Sarstedt	Not enough plasma to detect viral load	Design flaw with compartments	Fluid balance	3	3	4	36	Reexamine designs, run centrifuge for longer time
Impurities check	Plasma is full of impu-rities	Not pure enough to detect viral load	Design flaw with compartments, not separated for long enough	None	4	2	5	40	Reexamine designs, run centrifuge for longer time