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THE DIAMOND HEMESEEP BLOOD PROCESSING UNIT: A REAL-TIME MICROFLUIDIC WHOLE BLOOD SEPARATION PROCESS

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THE DIAMOND HEMESEP BLOOD PROCESSING UNIT: A REAL-TIME MICROFLUIDIC WHOLE BLOOD SEPARATION PROCESS

Abstract

Recent advancements in the field of microfabrication and microfluidics have made possible the design of separation devices and clinical diagnostic kits that use relatively smaller volumes of sample material than existing technologies. Using this technology, as well as existing technologies in membrane and immunomagnetic separations, a novel blood processing unit based on microfluidics has been designed. This report will detail the operation and layout of a microfluidic chip that produces three outputs (serum, plasma and a white blood cell lysate) from a human whole blood input. Microfluidic technology has allowed for the design of several distinctive features that make the performance of the blood processing unit comparable to existing centrifuge technologies available clinically and in research laboratories. Among other features, the chip produces a stabilized white blood cell lysate and is designed to match the blueprint of existing 96-well plates. In addition to describing the on-board processes and features of the chip, this report will also discuss the components needed for operation of the chip as well as a process to manufacture the product.

This product, known as the Diamond HemeSep blood processing unit, could offer more standardized, efficient blood separation technologies that would benefit health care providers, patients and researchers. Moreover, the product is predicted to have a healthy financial outlook: based on the target market of clinical laboratories performing preclinical and clinical trials involving numerous samples of blood, we expect to sell 1 million cartridges in the first year of production with sales growing to 1.7 million cartridges in the tenth and final year. The net present value (NPV) of the proposed project, based on a selling price of \$25 a cartridge, is expected to be \$51 million. For the current projections, Series A investors can expect returns of 45%.

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March 22, 2012

Professor Leonard Fabiano
Dr. Scott L Diamond
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Dear Professors Fabiano and Diamond,

Our group was presented with the task of designing a system, composed of a cartridge and processing unit that automatically processes a sample of citrated human whole blood. We succeeded in designing a device that provides three output fraction tubes consisting of at least 100 μ L blood plasma, 100 μ L serum, and a white blood cell lysate containing at least 10 μ g DNA within 30 minutes. We have named our product the "Diamond HemeSep Cartridge." Compared with existing products having similar functions, our product proves to be less expensive, faster, easier to use and requires less starting volume of whole blood to provide the same results.

The Diamond HemeSep outputs three different blood fractions: serum, plasma and a white blood cell lysate. The total market penetration is expected to be 50% after 3 years of total clinical trials for a blood processing market space of 1000 clinical sites in the country that process 1 million blood samples per year. With a price of \$25 per chip, our project gives a net present value (NPV) of roughly \$51 MM over 10 years, suggesting a profitable project.

Yours sincerely,

Daniel Moonan

Chinmay Paranjape

Jack Tirone

Kristina Wang

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Senior Design Reports (CBE)

University of Pennsylvania

Year 2012

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Chapter 1: Abstract

Recent advancements in the field of microfabrication and microfluidics have made possible the design of separation devices and clinical diagnostic kits that use relatively smaller volumes of sample material than existing technologies. Using this technology, as well as existing technologies in membrane and immunomagnetic separations, a novel blood processing unit based on microfluidics has been designed. This report will detail the operation and layout of a microfluidic chip that produces three outputs (serum, plasma and a white blood cell lysate) from a human whole blood input. Microfluidic technology has allowed for the design of several distinctive features that make the performance of the blood processing unit comparable to existing centrifuge technologies available clinically and in research laboratories. Among other features, the chip produces a stabilized white blood cell lysate and is designed to match the blueprint of existing 96-well plates. In addition to describing the on-board processes and features of the chip, this report will also discuss the components needed for operation of the chip as well as a process to manufacture the product.

This product, known as the Diamond HemeSep blood processing unit, could offer more standardized, efficient blood separation technologies that would benefit health care providers, patients and researchers. Moreover, the product is predicted to have a healthy financial outlook: based on the target market of clinical laboratories performing preclinical and clinical trials involving numerous samples of blood, we expect to sell 1 million cartridges in the first year of production with sales growing to 1.7 million cartridges in the tenth and final year. The net present value (NPV) of the proposed project, based on a selling price of \$25 a cartridge, is expected to be \$51 million. For the current projections, Series A investors can expect returns of 45%.

Chapter 2: Introduction

2.1 Introduction

Human whole blood is a complex bodily fluid that delivers oxygen and nutrients to the body's other organs and tissues. Whole blood is composed of red and white blood cells suspended in liquid called plasma (as described in detail in Chapter 5). Ordinarily, blood is separated into fractions of red blood cells, white blood cells and plasma through centrifugation, which takes advantage of the difference in buoyant densities of these different fractions. Plasma is then further purified into serum by chemically precipitating out the clotting factors. These various blood fractions are useful for analysis in many clinical and research settings. Knowledge about an individual's ion levels, coagulation system and a full DNA profile from white blood cell lysate can be useful for appropriate treatment and research during and following surgery, infection, or for other forms of diagnostics and therapies.

The Diamond HemeSep cartridge, as described in this report, presents an innovative design for blood processing and separation. Our cartridge uses advancements in microfluidics, immunomagnetic separation and filtration to provide a stabilized white blood cell lysate, a plasma fraction and a serum fraction as outputs in a small, self-contained package. With the aforementioned features, the cartridge's performance should be comparable to traditional blood processing technologies such as centrifugation.

Traditional blood processing has been performed using centrifugation (as described in Chapter 3). However, this procedure introduces unnecessary and inconvenient variability between samples dependent on the human operator conducting the analysis at the time. Furthermore, this procedure requires relatively large sample volumes and can be time consuming. Especially in analyses following a surgery or trauma, more immediate results are

often desired. In developing the Diamond HemeSep cartridge, we have addressed these concerns by standardizing the separation process and removing the need for consistent human presence during the separation procedure while maintaining the reliability and stability of existing technologies.

The Diamond HemeSep blood processing unit will initially be marketed for use in clinical research facilities that require blood to be processed for subsequent analysis. In such a clinical setting, the product may improve reproducibility of results and reduce waiting times in the labs, ultimately allowing for better patient care and scientific progress. It will do so by allowing physicians and researchers to acquire information that may be useful from their blood samples in a more timely fashion.

Chapter 5 describes the basic biology of human whole blood and briefly discusses the relevant chemistry of blood coagulation for the purpose of serum filtration from blood plasma, respectively. This chapter also discusses technologies currently in existence to fractionate blood and prevent and produce clotting. The Diamond HemeSep cartridge will produce the same blood fractions that current centrifugation technology can produce as well as further processing the white blood cell fraction to a DNA stabilized cell lysate. A novel benefit of the cartridge is the ability to produce and process all fractions of blood from a small starting sample volume simultaneously at the end of the processing time. This means that less blood needs to be collected from the patient and physicians and researchers do not have to wait for the samples to be processed in a laboratory by a human technician.

Chapter 6 introduces the scientific background information on the microfluidic design presented in Chapter 4 for separating plasma from the other blood components. While this method is not commonly in use, it is capable of producing nearly pure plasma fractions using

microliter scale samples of blood. The method works based on the Zweifach-Fung effect, which predicts that blood, when flowing through a main channel into a bifurcation, will fractionate into a cell fraction and plasma fraction based on the ratio of flow in the two daughter channels. For reasons described in Chapter 6, the blood cells will tend to flow into the larger channel with the lower flow rate. Chapter 9 discusses the manufacture of the microfluidic channels using a technique known as soft lithography.

Chapter 7 introduces the use of an antibody based system for white blood cell isolation from whole blood. The technique is based upon the affinity of antibodies, proteins produced by the immune system, for their ligands (in this case, white blood cells). After binding to their substrate, the antibodies, which are connected to a paramagnetic bead, can be concentrated to the bottom of a well along with the bound white blood cells to facilitate separation. The selection of an antibody and a more detailed explanation of the technique are presented.

Filtration as a mechanism for separating blood serum from plasma is discussed in Chapter 8. In addition, this chapter discusses the basic chemical reactions involved in coagulating and clotting blood so that the clots may be removed from plasma to produce a serum fraction.

In chapter 4, we present the layout of the microfluidic portion of the Diamond HemeSep cartridge, the design of the immunomagnetic separation for white blood cell and the specifics of the filtration system for filtering plasma into serum. These details include the geometry and spatial location of the various components and separation units on the cartridge as well as process flow diagrams that detail scheduling for the liquid handling unit.

A process for the manufacture of the Diamond HemeSep cartridge is outlined in Chapter 10. The microfluidic chips will be designed and prototyped internally, but mass produced

through an external vendor. However, the chips must be assembled onto the cartridge along with the tips and reagents needed for the other separation processes prior to packaging and shipping. Integral to proper performance of the cartridge is ensuring that the appropriate microliter volumes of sample and reagent can be delivered to their proper locations. To accomplish this, a liquid handling system will be used. While other engineering specialties, such as mechanical engineering, will need to be consulted to further develop this processing/handling tower, we have provided estimates for size and cost of the equipment needed. The manufacture of this tower as discussed in Chapter 10 will be outsourced to an experienced liquid handling company with revenues coming from both cartridge and unit sales.

After outlining these design considerations, Chapter 11 briefly elaborates on a proposed development timeline for the product. Integral to the success of any such medical device is acquiring FDA approval. The financial analyses presented in Chapter 12 will discuss the sensitivity of product success on delays in FDA approval.

Finally, the financial analysis examines numerous other scenarios, including the effects of varying product price and market penetration. Using an assumed selling price of \$25 per one time use cartridge, the NPV of the product is calculated to be roughly \$51 M. With further market research, product sales could grow beyond the assumed values presented herein.

2.2 Project Charter and Scope

Project Name	Microfluidic Blood Processing Unit
Project Champions	Scott Diamond, PhD
Project Leaders	Dan Moonan, Chinmay Paranjape, Jack Tirone, Kristina Wang
Specific Goals	Develop a microfluidic blood processing unit that can separate a 5mL sample of whole blood into plasma, serum, and white blood cells in 20 minutes.
Project Scope	<u>In-scope:</u> -Basic design of the disposable microfluidic cartridge -Define separation processes -Manufacturing procedure -Economic analysis -Experiment with designs of microfluidic chip
Deliverables	Business opportunity Market expansion Technical feasibility Manufacturing capability assessment Competitive product analysis Laboratory data analysis
Timeline	-The project feasibility and design stages will take place over the course of approximately 3 months

2.3 Purpose of Processing Blood

Preclinical and clinical trials often involve the collection and processing of blood to obtain erythrocyte, leukocyte, and platelet cell counts, as well as stable serum and plasma samples. Techniques such as proteomics, metabolomics, and DNA analysis often involve assays and require efficient isolation of specific blood components. Plasma and serum are harvested and stored for analysis of analytes, biomarkers, etc. As well, the leukocyte samples are often used for later preparation of DNA or for constructing DNA archives (1).

2.4 Innovation Map

An innovation map is used to address the need for new technologies when preparing a new product. As discussed by Seider et al., an innovation map has six levels. Listing these levels from top to bottom, they are: customer-value proposition, products, product technology, technical differentiation, process/manufacturing technology, and materials technology. The map connects these levels by stating which new technological features will be used in the development of the product (1, 2).

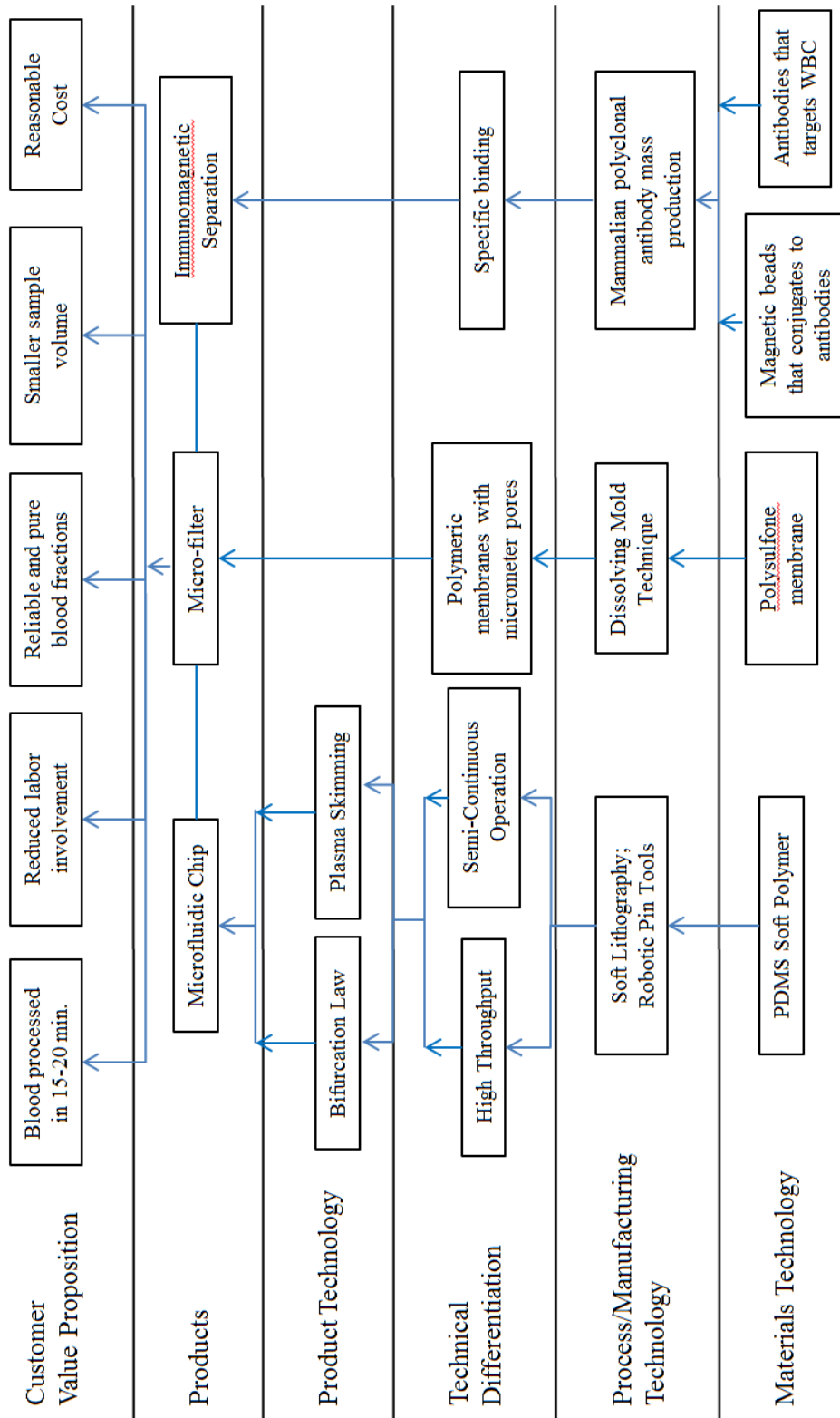


Figure 2.1: Diamond HemeSep's

Chapter 3: Market Analysis

3.1 Markets for the Diamond HemeSep

As directed in the design statement, the Diamond HemeSep will initially be marketed toward use in pre-clinical and clinical trials. The Diamond HemeSep will offer significant value to the research community by eliminating inefficiencies in current practices of processing blood. Our initial target customers are pharmaceutical companies, biotechnology research and development companies, and hospital and clinical laboratories. The size of this market has been estimated based on the number of clinical sites and blood samples. As outlined in the design statement, we assume a market space of 1000 clinical sites that process 1 million blood samples a year in the U.S.

We have also identified other potential applications of the Diamond HemeSep. During product prototyping, entrance into the other blood markets described below should be considered to explore the possibility of significantly increasing revenue.

Aside from research purposes, quantification of components in blood, such as leukocytes, platelets, and serum proteins, is routine for the diagnosis and monitoring of many diseases. For example, quantification of serum proteins is used as a diagnostic test for diseases such as paraproteinaemias, hemoglobinopathies, and genetic abnormalities (2). The Diamond HemeSep may allow physicians and scientists to exercise point-of-care diagnosis and acquire information in a timely manner. Furthermore, in many surgeries, especially cardiac surgeries undergoing cardiac pulmonary bypass (CPB), there is an unmet medical need to monitor inflammation by fractionating blood and measuring the concentration of clinically relevant proteins (1-3). Exposure of blood to non-physiological surfaces of the cardiopulmonary bypass, hypothermia, surgical trauma, and ischemia-reperfusion of the involved tissues induces complex inflammatory responses and are considered as main factors causing postoperative complications. These complications include vital organ dysfunction that can lead to multi-organ failure and even death. The intensity of the inflammatory response appears to be directly correlated with the severity of CPB-related

morbidity. Currently, there is no effective method for preventing this systemic inflammatory response syndrome in cardiac surgery patients undergoing CPB. Therefore, the Diamond HemeSep can potentially fulfill this unmet medical need by offering a safe and effective therapeutic diagnostic to monitor the inflammatory response in surgeries.

Thus, in addition to the research market, the Diamond HemeSep may be applicable to various diagnostic markets. Our other potential targeted customers include physicians' offices, nursing homes, and surgery operating rooms, where access to a clinical laboratory is limited.

3.2 Customer Requirements

Considering the needs and features required by potential customers is crucial to designing a new product and will most likely determine whether the product succeeds or fails. Customer requirements are determined by analyzing data from the market survey and researching competing products. Once a list of customer requirements is compiled, each requirement is given a weighting factor to designate its degree of importance and is also classified as either *fitness-to-standard* (FTS) or *new-unique-difficult* (NUD).

Table 3.1 shows the desired customer requirements (2).

Customer Requirement	Product Requirements	Type	Weighting Factor (%)
Pure blood fractions	Instrument/measurement quality	FTS	20
Reproducible blood fractions	Instrument/measurement quality	NUD	20
Minimization of labor involvement	Automation	NUD	15
Faster processing time	Automation	NUD	15
Low whole blood input volume	Low whole blood input volume	FTS	5
Portability	Instruments that occupy least space	FTS	10
Low cost	Cost-effective separation method	FTS	15

Table 3.1: Customer requirements

3.2.1 Critical-to-Quality variables

The customer requirements from the previous section must be translated into technical requirements that can be manufactured and used in the design of the device. These technical requirements are also called critical-to-quality variables (CTQ) and relate to specific target values. The target values have been determined by researching competing products and industry standards (2, 6).

Product Requirement	Technical Requirement (CTQ)	Target
Instrument/measurement quality	Liquid Handling Robot Microfluidic chip Immunomagnetic Separation Microfilter Efficient Separation	~92-98% purity >80% yield
Automation	Microfluidic chip Liquid Handling Robot	Processing time within 30 min.
Low whole blood input volume	Microfluidic chip	<10mL
Instruments that occupy least space	Microfluidic chip Cartridge Liquid Handling Robot	No larger than desktop computer tower
Cost-effective separation method	Microfluidic chip Immunomagnetic Separation Microfilter	Costs under \$25

Table 3.1: Critical-to-Quality variables

3.3 Competition in the blood processing market

Conventional plasma and leukocyte separation methods have relied on membrane-based filtration and centrifugation. Membrane-based filtration uses hydrostatic pressure to force a liquid containing the biomolecule mixture against a semi-permeable membrane (2, 4). However, due to high cellular fractions in blood, membrane-based filtration leads to clogging and compromise separation efficiency.

Centrifugation is the process that uses centrifugal force to isolate solid suspended particles from their surrounding liquid media (2, 4). To separate macromolecules such as proteins and DNA, the solution usually runs in a special medium that separates into distinct density zones. Traditional bench-top centrifuges are known to be expensive, time consuming and labor intensive. In an effort to realize centrifugation on a microscale, disk centrifuges use compact disk-like platforms with manifolds and a

spinning motor plate to achieve centrifugal pumping. However, during centrifugation the sedimented blood cells can easily lyse, thereby releasing intracellular components that contaminate the plasma sample (2, 4, 5). Microfluidics has the potential to overcome these limitations. Microfluidics is the science of studying fluid flow behavior at the microscale and the development of miniaturized analysis systems that take advantage of the unique physics at these small scales (2, 4). Microfluidics leverages its many distinct features such as low sample volume, reduction in processing time, automation of processing steps, and capability to produce reliable and selective outputs. These advantages make microfluidics an attractive separation method for point-of-care applications and laboratories with high throughput demands.

Currently, there are no microfluidic-based blood processing devices on the market. Our closest direct competitor is centrifuge-based blood processing machines, such as ones sold by RTS Life Sciences.

RTS Life Sciences ABF 200 is a centrifuge-based blood processing machine that automates the separation, storage, and tracking of blood samples. The machine has a proprietary signaling system that accurately measures the fraction heights of centrifuged blood in collection tubes. After calculating each volume using the dimensions of the tube, this information is transferred to a liquid handling robot to aspirate and dispense the fractions. The machine processes up to 500 samples a day in either 6ml or 10ml collection tubes and has dimensions of 2m x 2.4m x 1m (2, 4-6). Per quote from sales representative at RTS Life Sciences, the ABF 200 is typically sold at around \$700,000.



Figure 3.1 RTS Life Sciences ABF 200

3.4 Distinguishing Features of the Diamond HemeSep

A comparative analysis for the Diamond HemeSep and its competing products proves that the Diamond HemeSep is the superior product. The Diamond HemeSep delivers significant value to our

customers by saving time, labor, and money, while providing reproducible and high-quality results. Table 3.3 compares major features and prices of our device against competing products.

One main feature of the Diamond HemeSep is the automation of the process that eliminates labor and saves time. By leveraging the microfluidics technology and liquid handling, the Diamond HemeSep can separate whole blood into the three desired components in 24 minutes, which is significantly less than the 60 to 90 minutes under manual processing. Furthermore, automation eliminates the need for consistent human involvement in the process. This allows staff resources to be better utilized and reduces costs. Automation also minimizes the exposure to unscreened blood and thus reduces the health risks exposed to staff. Under manual processing, the separated blood fractions often have highly variable purity. Automation overcomes this limitation and, through standardization, gives reproducible and accurate outputs. These advantages ultimately results in greater productivity for our customers.

Other distinct features of Diamond HemeSep differentiate it from competing options and appeal to our target customers. Unlike the competing products, the Diamond HemeSep only requires small volume of whole blood input while still achieving efficient separation. This feature is highly desirable in clinical trials where blood samples are rare, such as in the fields of neonatology and orphan diseases. As well, the Diamond HemeSep is highly portable and offers the convenience of a relatively small device, since it does not occupy more space than a desktop computer tower. Unlike competing devices, such as clinical laboratory centrifuges or the competing product ABF 200, the Diamond HemeSep can be used in new locations such as physician's offices that have limited access to clinical laboratory facilities. This will allow healthcare professionals to acquire information in a timely manner. This feature is also highly desirable in laboratories where space is limited and real estate costs are high. Moreover, the price of our product is also significantly lower than the prices of competing products.

	Centrifuge	ABF 200	Diamond HemeSep
Exposure to Health Risks	Technicians exposed to unscreened blood	Minimized	Minimized
Processing time	~60-90 min. per 10mL whole blood	A rack of 24 vacutainers in <5min.	~24 min. per 5mL whole blood
Labor	Labor intensive; lots of waiting time	Automated	Automated
Consistent/Reproducible results	No	Yes	Yes
Quality results	No	Yes	Yes
Space	Equipment and technicians; occupies most space	2m x 2.4m x 1m	4" x 6" x 3"; occupies least space
Sample Volume	>10ml	6ml or 10ml	<5ml
Cost	Centrifuge: ~\$5000 Labor Costs: significant	>\$700,000	Cartridge: <\$25 Processing Unit: \$100,000

Table 3.3: Quality and price comparisons between the Diamond HemeSep blood processing unit and the ABF 200

3.4.1 House of Quality

The House of Quality (HOQ) relates the customer requirements to the overall product requirements and consists of six sections. The first section is a list of the customer requirements and the second section lists the technical requirements associated with the customer requirements. The third section consists of a matrix that shows the relationships between the customer and technical requirements, showing whether or not the technical requirement exists for a certain customer requirement. The fourth section, or the top of the house, shows the synergies and conflicts among the technical requirements. In this section, a plus sign is used to show synergies between both variables while a minus sign is used to show conflicts between both variables. If no relationship exists between the variables, the space is left blank. The final section displays the weighting factors for the customer requirements which were already determined in the customer requirements table (2).

					1	<i>Efficient Separations</i>
					1	<i>Liquid Handling Robot</i>
			1		+	<i>Immunomagnetic Separation</i>
		1			+	<i>Micro-filter</i>
	1				+	<i>Microfluidic Device</i>
<i>Customer Requirement</i>	<i>Microfluidic Device</i>	<i>Micro-filter</i>	<i>Immunomagnetic Separation</i>	<i>Liquid Handling Robot</i>	<i>Efficient Separations</i>	<i>Weight (%)</i>
<i>Pure blood fractions</i>						20
<i>Pure plasma fraction</i>	x				x	
<i>Pure serum fraction</i>		x			x	
<i>Pure WBC lysate</i>			x		x	
<i>Consistent blood fractions</i>						20
<i>Consistent plasma fraction</i>	x				x	
<i>Consistent serum fraction</i>		x			x	
<i>Consistent WBC lysate</i>			x		x	
<i>Minimization of labor involvement</i>	x			x		15
<i>Faster processing time</i>	x			x		15
<i>Low whole blood input volume</i>					x	5
<i>Portability</i>	x			x	x	10
<i>Low cost</i>	x	x	x	x	x	15

Figure 3.2: Diamond HemeSep Cartridge’s House of Quality Matrix

3.5 Market Projection

All revenues in the near future of the Diamond HemeSep will come from its focus on the pre-clinical and clinical trials market. In the future, other possible markets such as disease diagnostic or inflammation monitoring in surgeries will be studied. At present, sales in other markets will not be considered. It has been estimated that there are 1000 total clinical sites in the U.S. and that 1 million blood samples are processed each year. It was also assumed that the number of clinical sites and blood samples will grow at a rate equivalent to the growth rate of the blood industry. The average growth rate of the blood collection and processing industry is 5.5% (37).

Following development of the product, we have assumed that sales of the Diamond HemeSep can be maintained for 9 years. Diamond HemeSep's market share is approximated to be 15% in the first year of production, growing to 30% in the second year of production, and reaching 50% in the third year of production. It is assumed that Diamond HemeSep will maintain this market share in the rest of production years. While this kind of market penetration seems ambitious, we believe that it is achievable due to the quality of our product and the nature of the market. We expect that the Diamond HemeSep's automation of blood processing and its other features will provide significant value to clinical laboratories. Since the technology of our device is novel, there should be little competition. As scientists and healthcare professionals recognize the value of our product, the use of microfluidic-based blood processing devices could become a standard within the industry.

Based on these projections, the number of single-use Diamond HemeSep Cartridges we expect to sell ranges from about 160,000 in 2014 to 900,000 in 2023. Assuming 250 days of production annually and eight hour work days, manufacturing requirements will range from 80 to 450 cartridges per hour.

Sales for the processing unit were estimated based on assumptions that the processing unit has a product life of 10 years and that each clinical site uses one unit. Additional revenue from maintenance and repair services is estimated as 15% of revenue from sales of the processing units.

3.6 Patents

The intellectual property of the Diamond HemeSep will be limited primarily by patents held by Bayer Healthcare LLC. Bayer Healthcare's patent US 7094354 B2, filed on December 19, 2002, describes the separation of particles using a microfluidic device (38). This should pose no major obstacles because the Diamond HemeSep will file new patents of novel designs of the microfluidic chip and the cartridge.

3.7 Summary

Overall, our market analysis showed that the Diamond HemeSep is an innovative technology with the potential to disrupt the blood processing industry. There is a significant unmet need for a more efficient and reliable method to fractionate blood. By leveraging its microfluidic technology and automation process, the Diamond HemeSep can fulfill this need. Currently, there are no microfluidic-based blood processing devices on the market. Thus, the Diamond HemeSep has the potential to realize first-mover advantages. Moreover, since it is portable and relatively low cost, the Diamond HemeSep offers significant value over competing products. Under base case assumptions, we expect to achieve sales of up to 900,000 single-use cartridges per year. Additional revenue streams include sales of the processing unit and maintenance and repair service fees.

Our business model will initially be targeted at the research and clinical trials market. We will target pharmaceuticals, biotech research and development companies, and hospital and clinical laboratories. Since the technology of the Diamond HemeSep is theoretically applicable for diagnostic uses as well, it may be possible to expand into other diagnostic markets for various diseases.

Chapter 4: Diamond HemeSep Cartridge

4.1 Process Overview

4.1.1 Cartridge Diagram

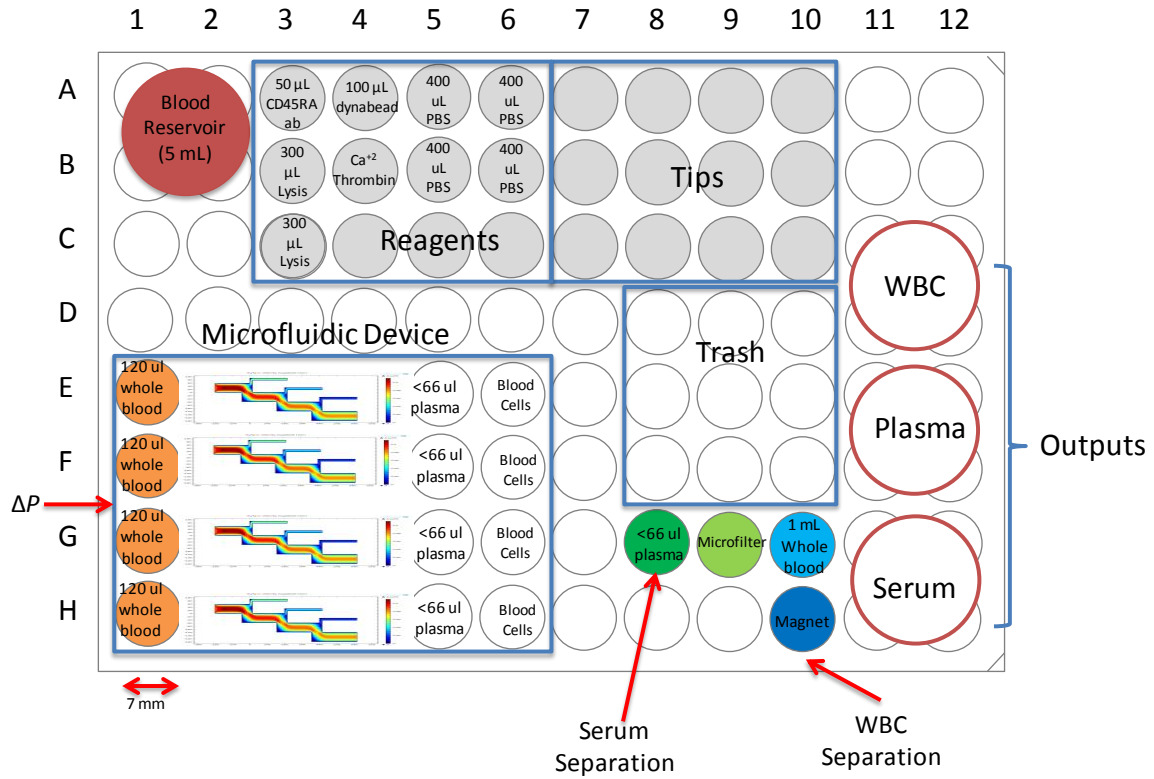


Figure 4.1a: 2-D diagram of the Diamond HemeSep cartridge.

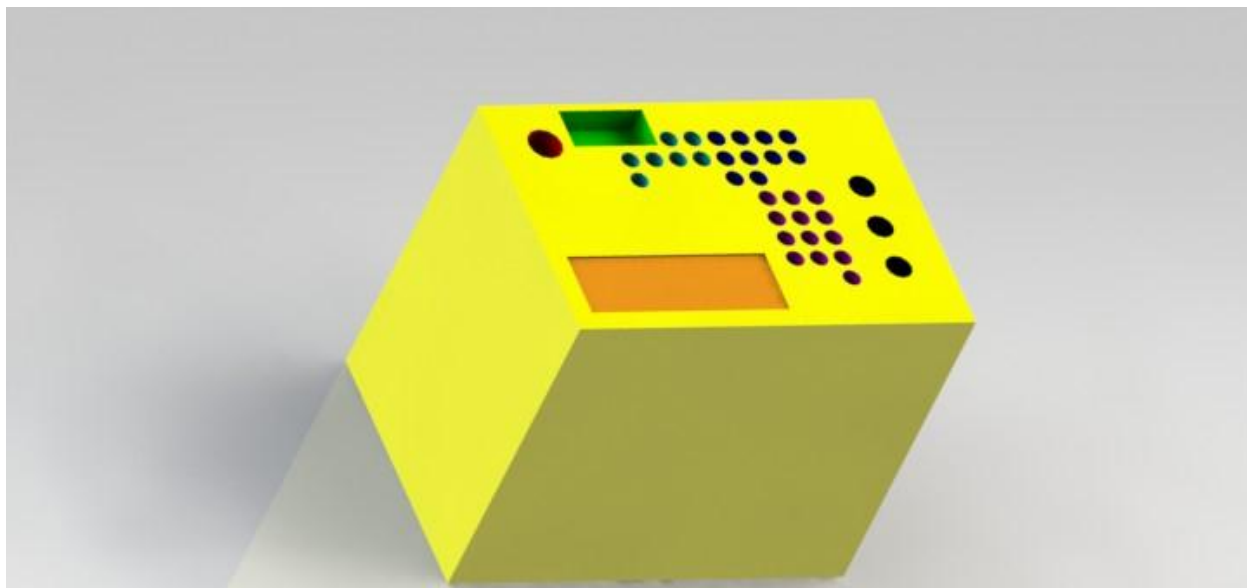


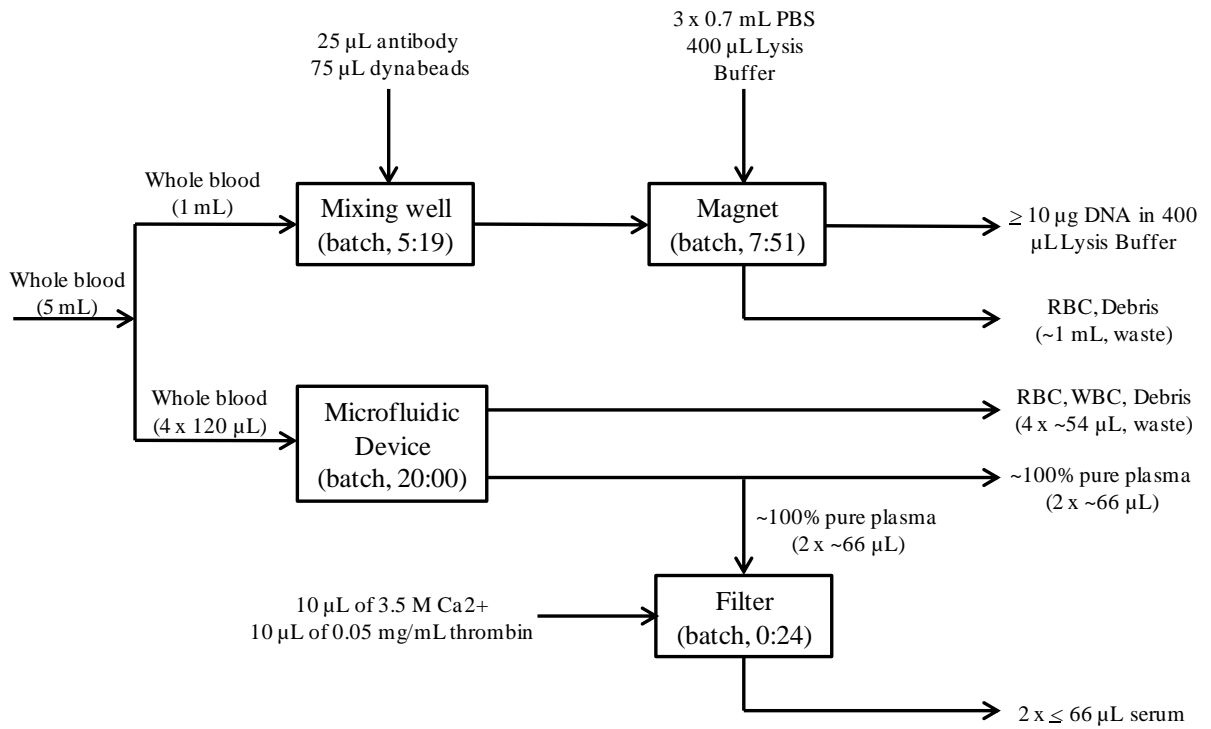
Figure 4.1b: 3-D rendition of the Diamond HemeSep cartridge.

Building on the principles outlined in coming chapters, this chapter describes the design of a cartridge to create a stabilized DNA lysate from WBCs, a plasma output and a serum output. We sought to design a system that emulates the reliability and reproducibility of clinical laboratory methods. To do so, we designed three separate, parallel separations processes that use microfluidics to separate plasma from whole blood, immunoprecipitation to isolate and then lyse WBCs from whole blood, and a filtration system to separate serum from plasma.

Figure 4.1a is a Microsoft PowerPoint schematic of our cartridge design. Figure 4.1b is a 3-D rendition of the cartridge developed in the CAD software Solidworks. The cartridge has been designed to the specifications of a standard 96 well microplate for the ease of integrating its use with existing liquid handling technologies. The patient's blood sample, collected in a 5 mL citrated vacutainer, is loaded into the red input labeled "Blood Reservoir" on the cartridge prior to processing. As described in later sections of the chapter, the original blood sample is initially aliquoted into samples for plasma separation and white blood cell separation. First, 1 mL of whole blood is placed in well G10, where the white blood cell processing steps, described later,

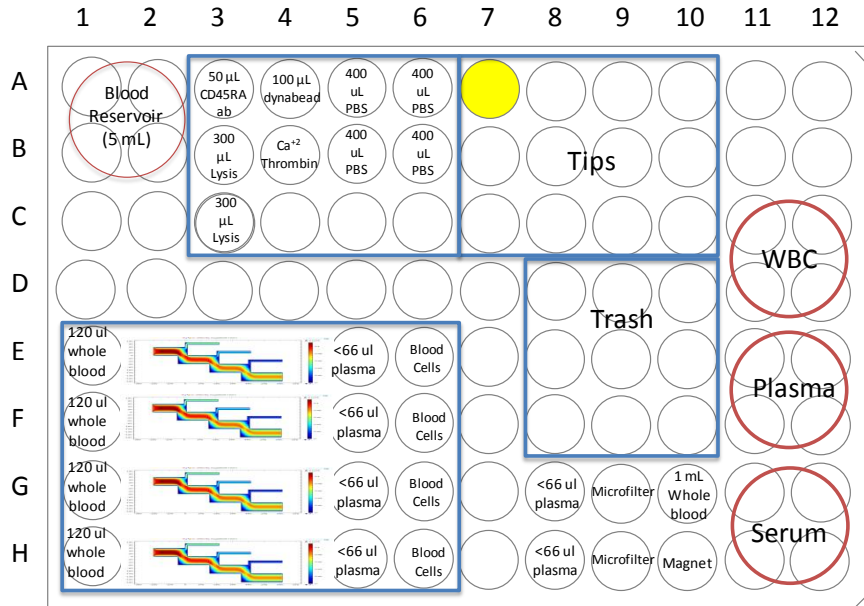
occur in wells G10 and H10 before a final output is created and stored in the larger circle labeled “WBC”. Somewhat simultaneously, the liquid handler withdraws two aliquots of 120 μL of whole blood from the original input and deposits the blood to wells E1 –H1. Then the liquid handling robot applies a constant pressure gradient (ΔP in Figure 4.1a) to maintain a blood flow rate of 10.5 $\mu\text{L}/\text{min}$ through each of the four microfluidic bifurcations. This occurs for a total of 20 minutes, with brief interruptions to perform mixing steps as detailed in the WBC processing section. The flow through the microfluidic chip separates the whole blood into a nearly pure plasma fraction in wells E5 – H5 and a waste cell, platelet and debris fraction in wells E6 – H6. This occurs through a phenomenon known as the Zweifach-Fung effect, described in Chapter 6. A more detailed CAD drawing of the microfluidic portion of the cartridge is provided later. After the first two aliquots of plasma have been processed, they are transferred to the serum processing section of the cartridge in well G8. After calcium and thrombin have been added as detailed further in section 4.2.2, and the plasma has been clotted, the microfilter in G9 is used along with the liquid handler to remove purified serum from the plasma fractions. The plasma and serum outputs will be stored in the large red circular areas labeled “Plasma” and “Serum” for the end-user.

4.1.2 Overall Process Flowsheet

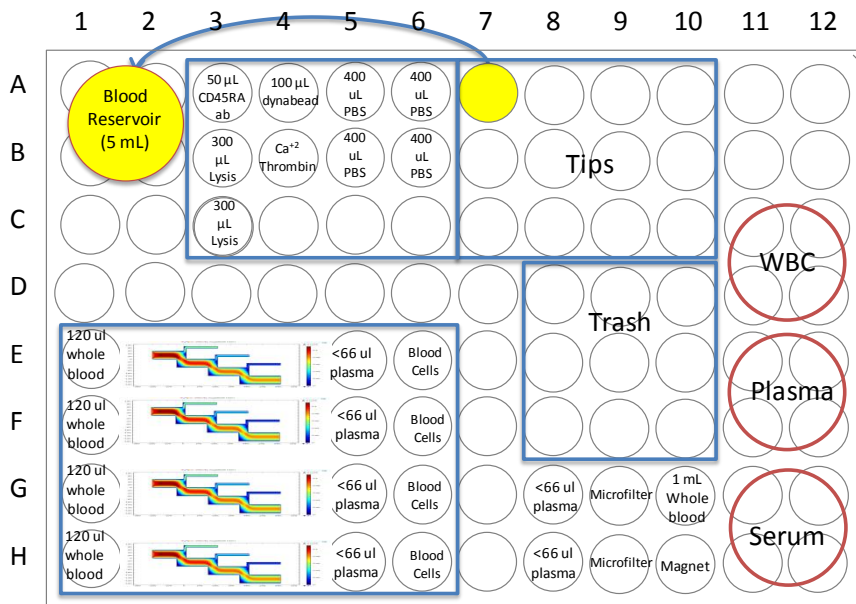


4.1.3 White Blood Cell Processing Flowsheet

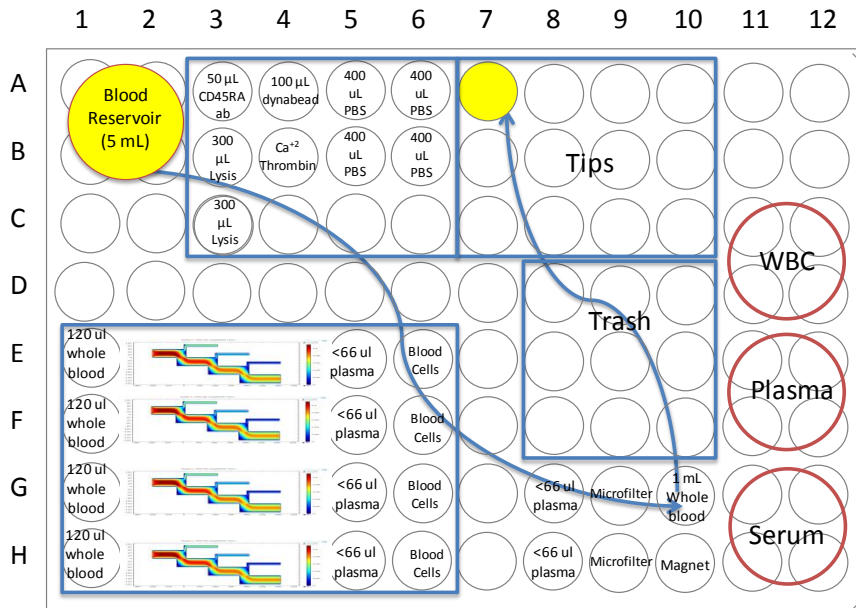
Step 1: Machine picks up 1st tip. Step information: (t = 2 s, tips = 1).
 Cumulative information: (t = 2 s, tips = 1)



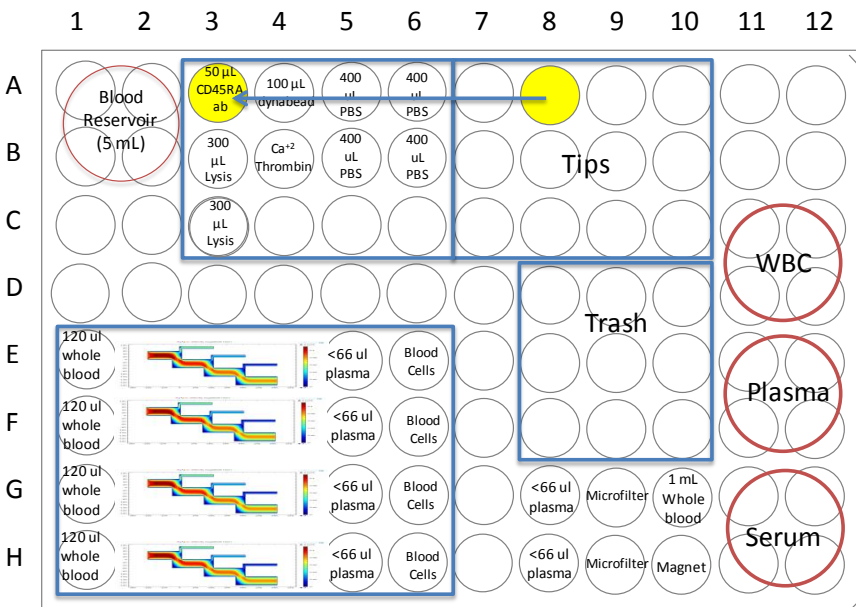
Step 2: Draws up 1 mL whole blood. Step information: (t = 2 s, tips = 0).
 Cumulative information: (t = 4 s, tips = 1)



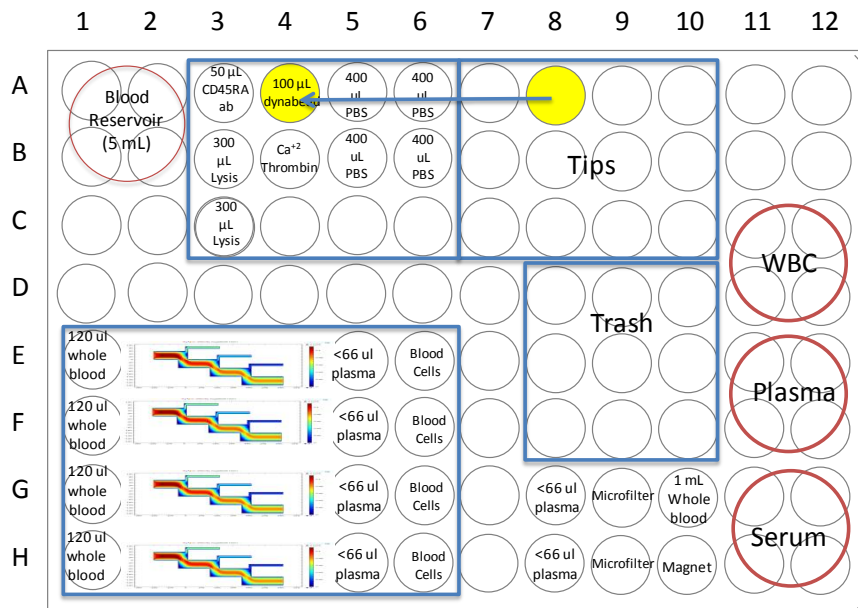
Step 3: Pipette 1 mL whole blood into 1st well, put tip back. Step information: (t = 5 s, tips = 0). Cumulative information: (t = 9 s, tips = 1)



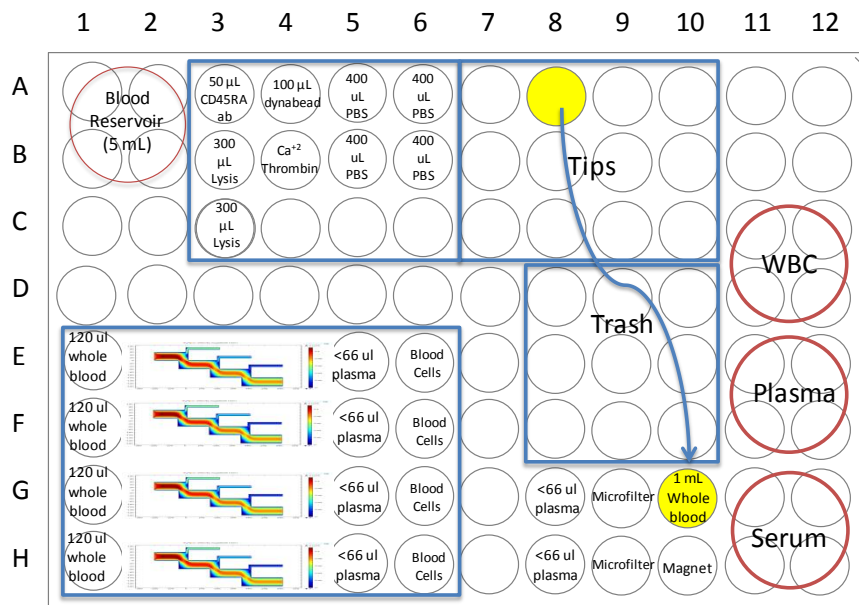
Step 4: Machine picks up 2nd tip, withdraws 25 µL FlowComp CD45 RA antibody. Step information: (t = 4 s, tips = 1). Cumulative information: (t = 13 s, tips = 2)



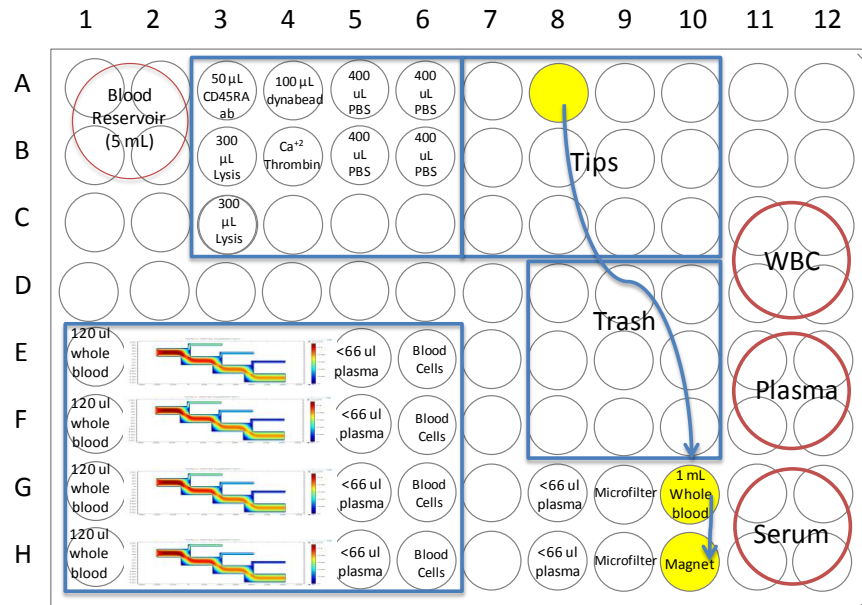
Step 5: Machine pipettes antibody into whole blood, picks up 75 μ L bead and pipettes into blood. Step information: (t = 6 s, tips = 1). Cumulative information: (t = 19 s, tips = 2)



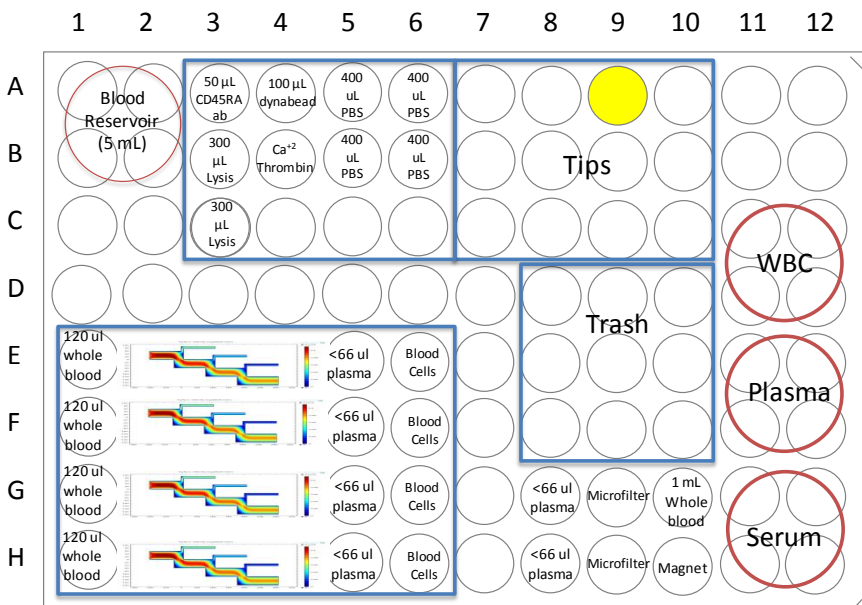
Step 6: Mix for 25 s with tip, put tip back and use hand for other processes, come back with same tip, repeat for 5 min total. Step information: (t = 5 min, tips = 0). Cumulative information: (t = 5:19 s, tips = 2)



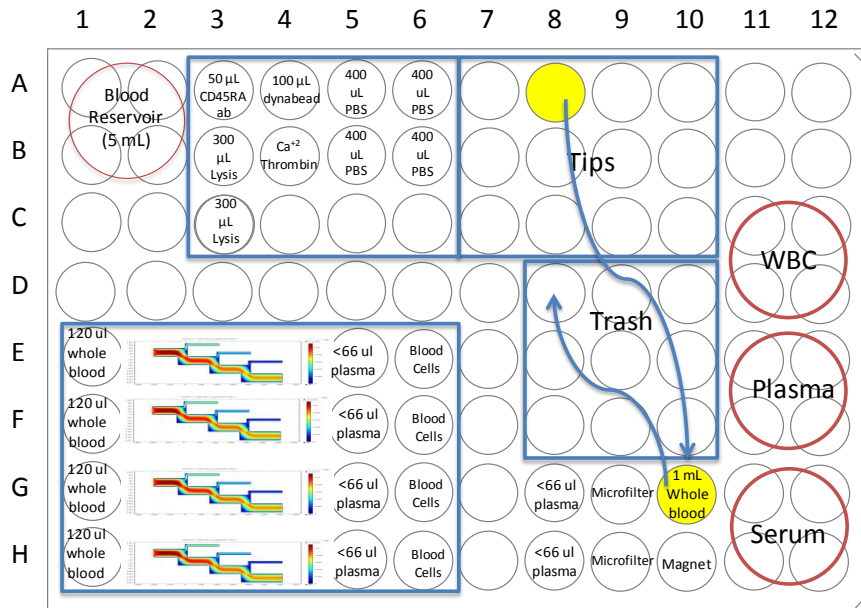
Step 7: Transfer bead, antibody, blood mixture to magnetic well using the same tip.
 Let magnet concentrate WBC for 2 min
 Step information: (t = 2 min, tips = 0). Cumulative information: (t = 7:19, tips = 2)



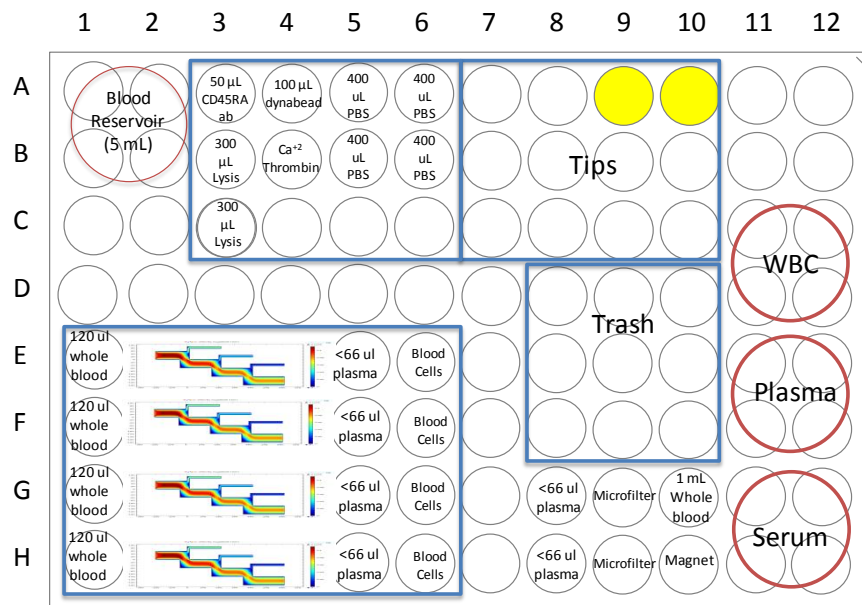
Step 8: Machine picks up 3rd tip.
 Step information: (t = 3 s, tips = 1). Cumulative information: (t = 7:22, tips = 3)



Step 9: Machine withdraws 0.7 mL supernatant and disposes in waste.
 Step information: (t = 6 s, tips = 0). Cumulative information: (t = 7:28, tips = 3)

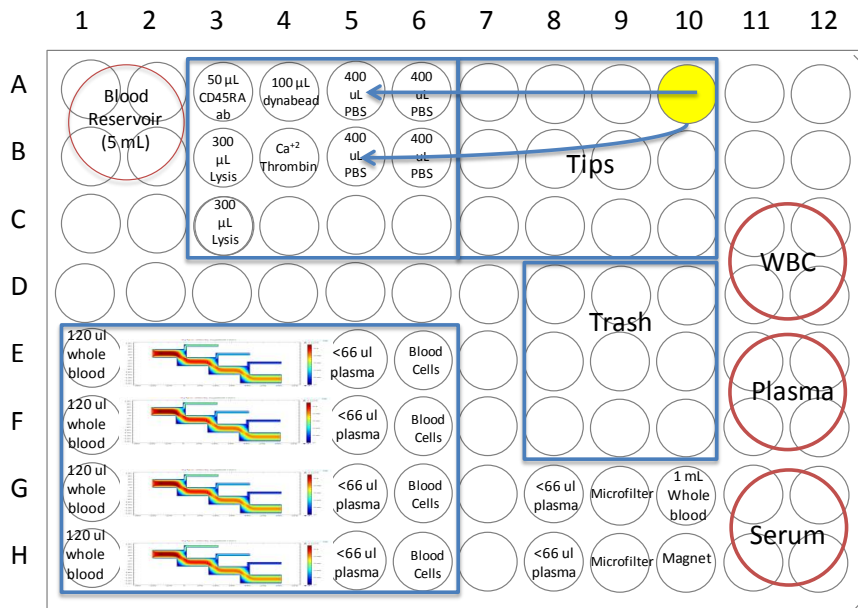


Step 10: Machine puts 3rd tip back, picks up 4th.
 Step information: (t = 5 s, tips = 1). Cumulative information: (t = 7:33, tips = 4)



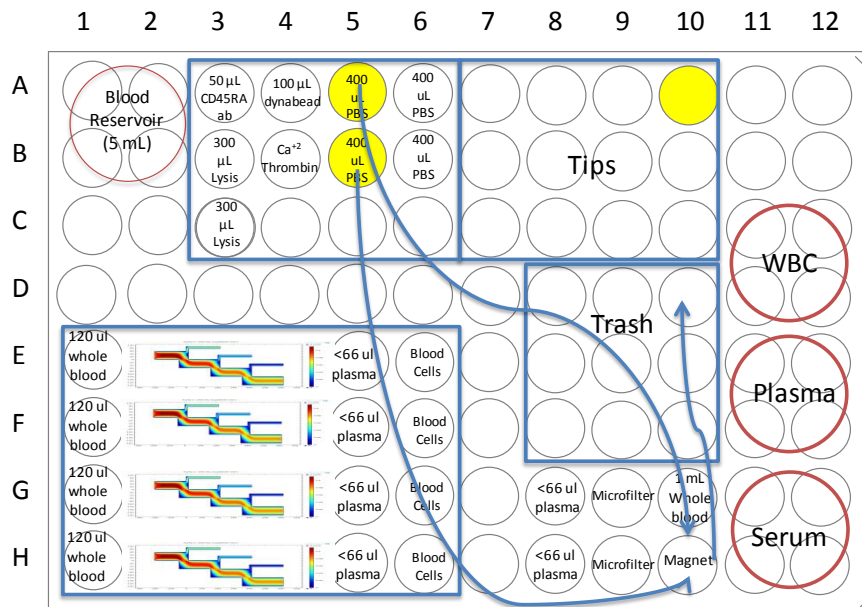
Step 11: Machine withdraws 0.7 mL PBS.

Step information: (t = 2 s, tips = 0). Cumulative information: (t = 7:35, tips = 4)

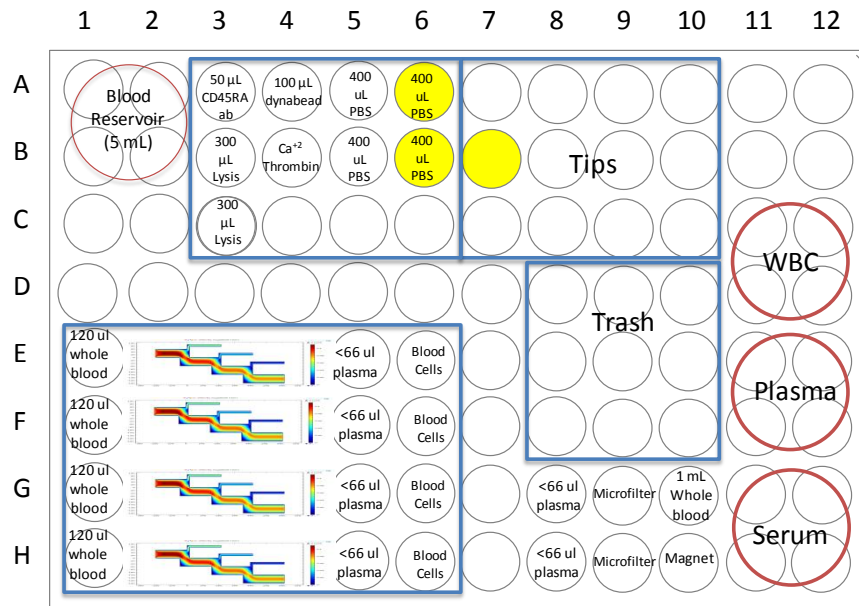


Step 12: Gently pipette up and down, move liquid (0.7 mL) to trash, put 4th tip back

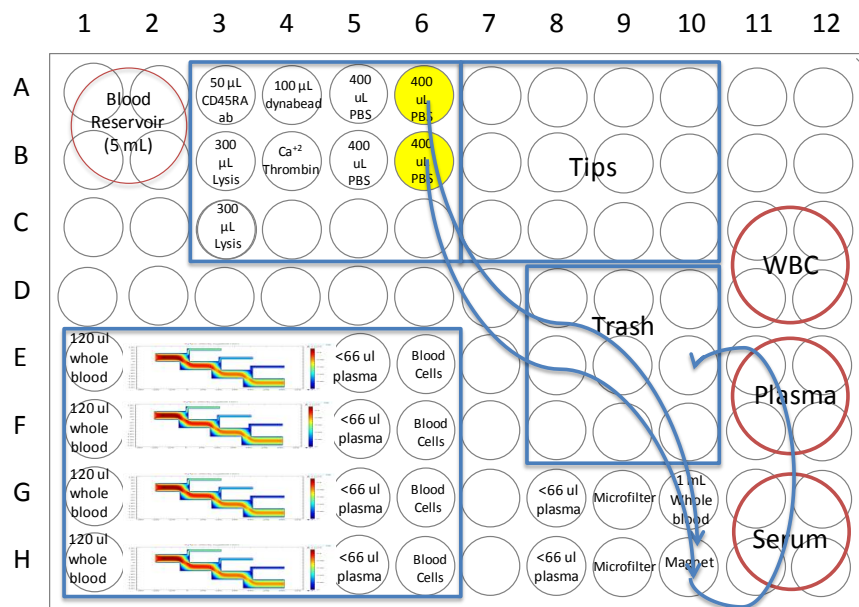
Step information: (t = 10 s, tips = 0). Cumulative information: (t = 7:45, tips = 4)



Step 13: Machine picks up 5th tip and withdraws 0.7 mL PBS
 Step information: (t = 5 s, tips = 1). Cumulative information: (t = 7:50, tips = 5)

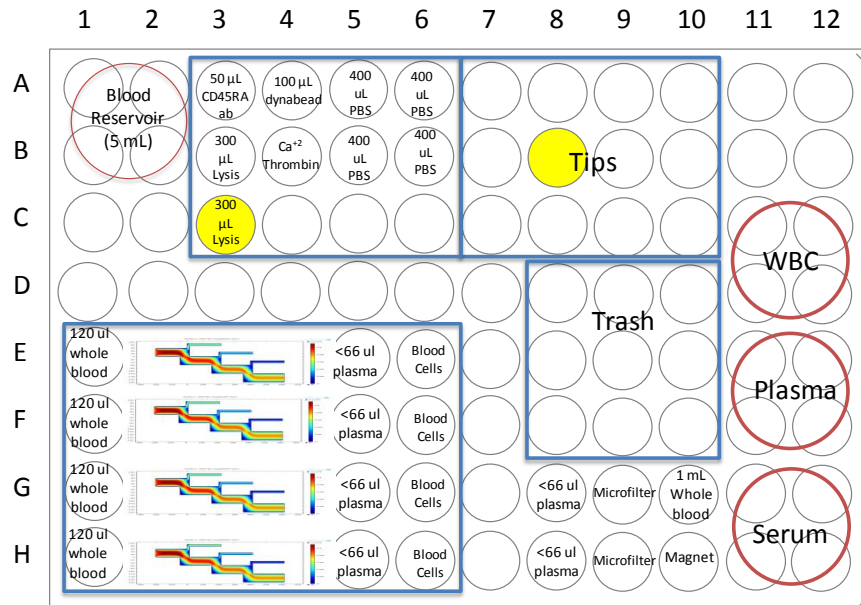


Step 14: Gently pipette up and down, move liquid (0.7 mL) to trash, put 5th tip back
 Step information: (t = 10 s, tips = 0). Cumulative information: (t = 8:00, tips = 5)



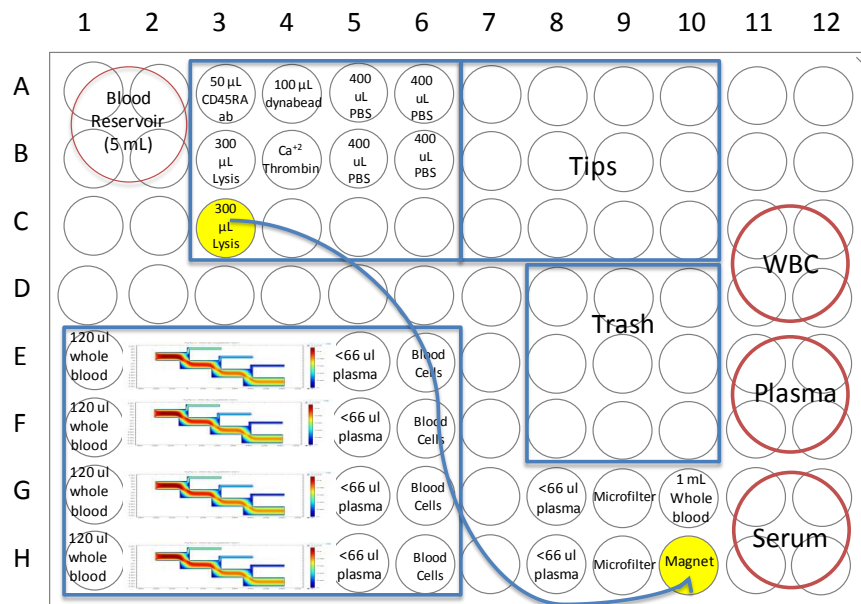
Step 15: Machine picks up 6th tip and withdraws 400 μ L 1X Lysis buffer (Cell Signalling Technologies)

Step information: (t = 5 s, tips = 1). Cumulative information: (t = 8:05, tips = 6)



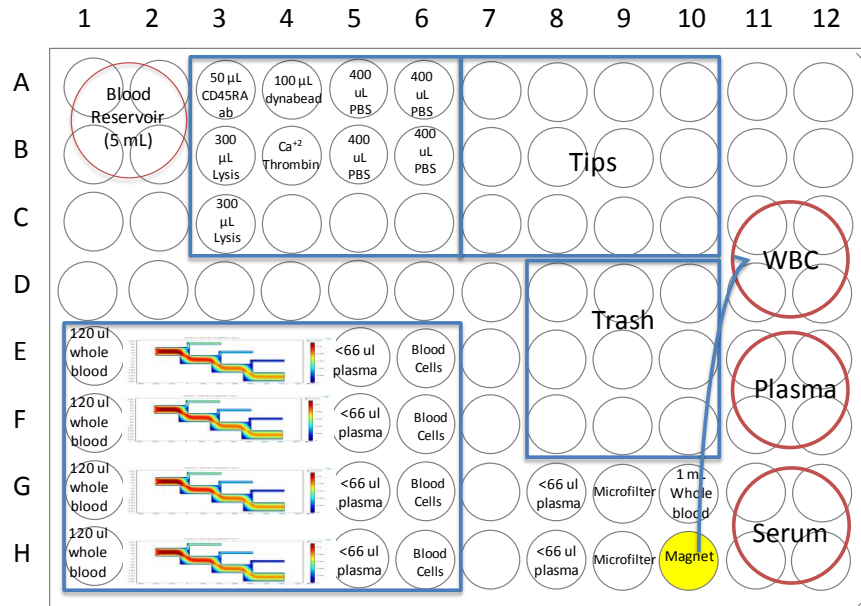
Step 16: Add buffer to cells and pipette mix for 30 s on / off for 5 minutes

Step information: (t = 5 min, tips = 0). Cumulative information: (t = 13:05, tips = 6)



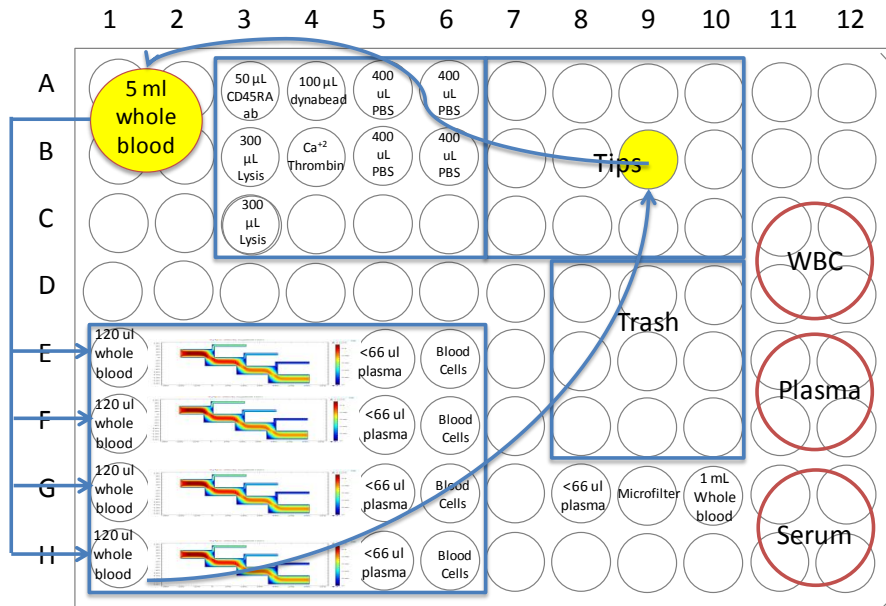
Step 17: Transfer cell lysate to WBC product well

Step information: (t = 5 s, tips = 0). Cumulative information: (t = 13:10, tips = 6)

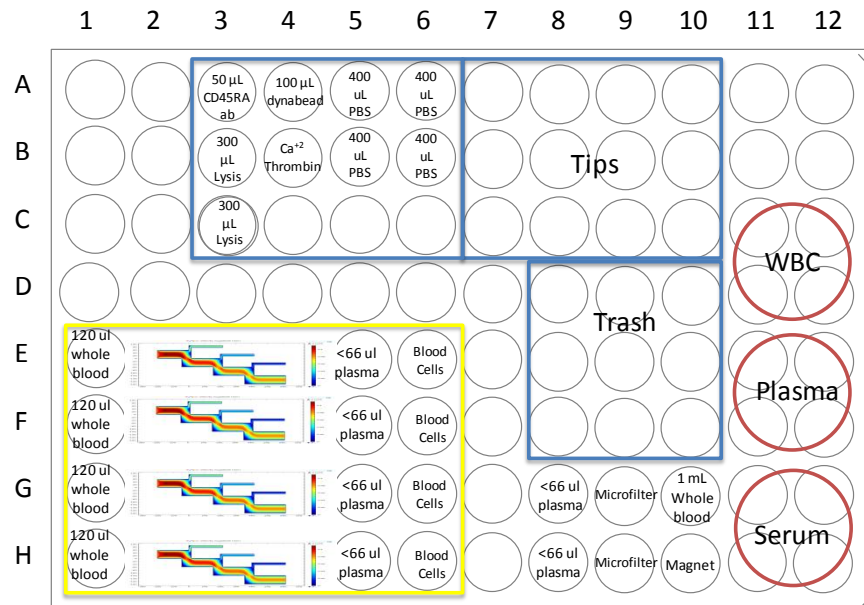


4.1.4 Plasma and Serum Processing Steps

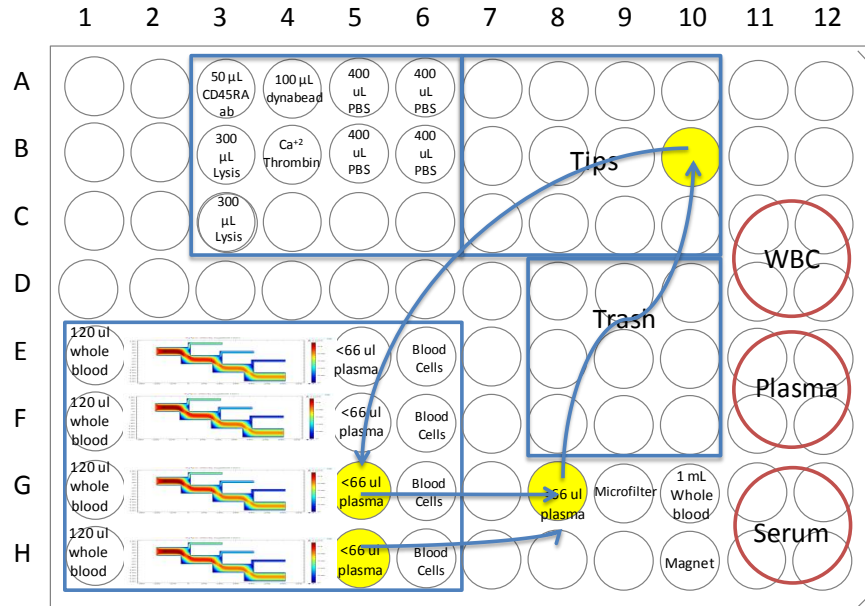
Step 1: Pick up tip, transfer 120 ul of plasma into 4 wells on microfluidic chip, and put tip back. Step information: (t = 18 s, tips = 0). Cumulative information: (t = 0:18, tips = 1)



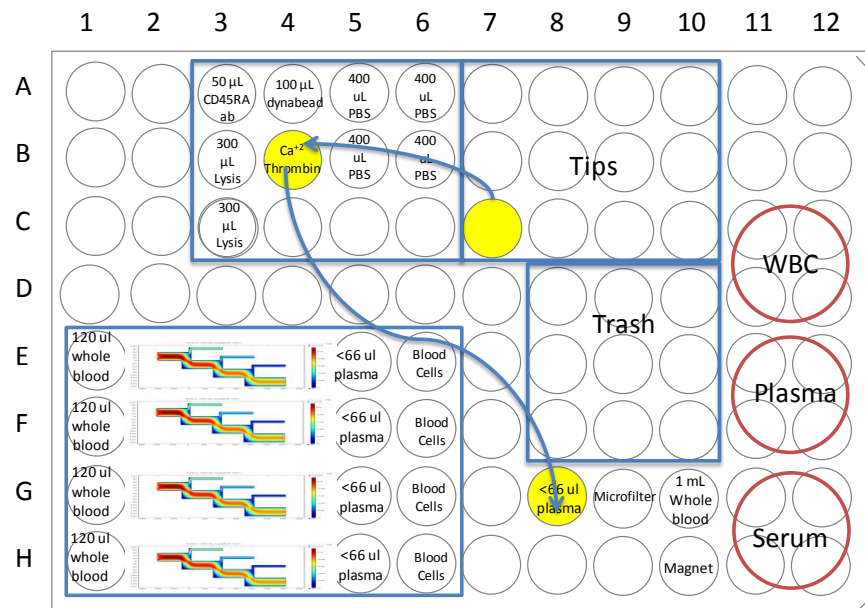
Step 2: Bifurcation Process – Run for total of 20 minutes at a flow rate of 10 μL/min. Step information: (t = 20:00, tips = 0). Cumulative information (t = 20:18, tips = 1)



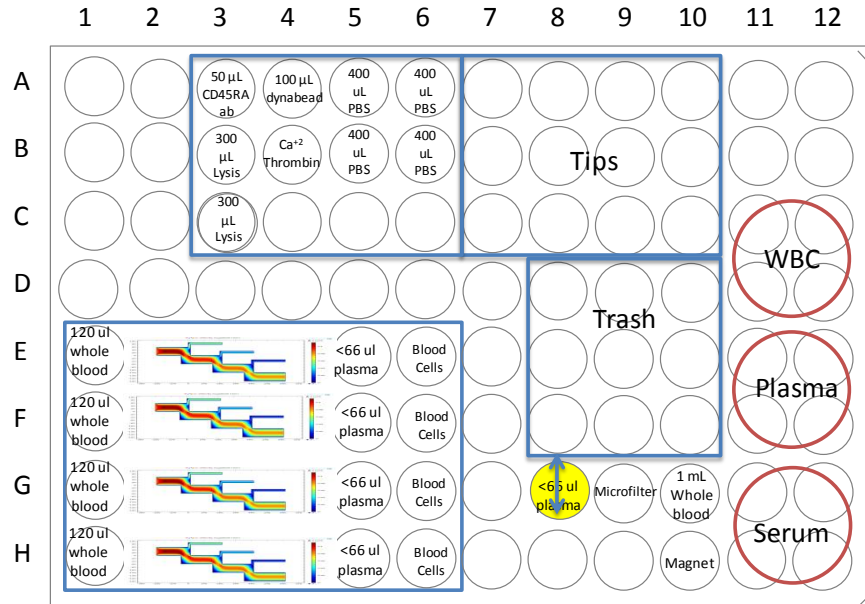
Step 3: Pick up tip, transfer <66µL plasma into well for serum separation, return tip.
 Step information: (t = 0:12, tips = 1). Cumulative information: (t = 20:30, tips = 2)



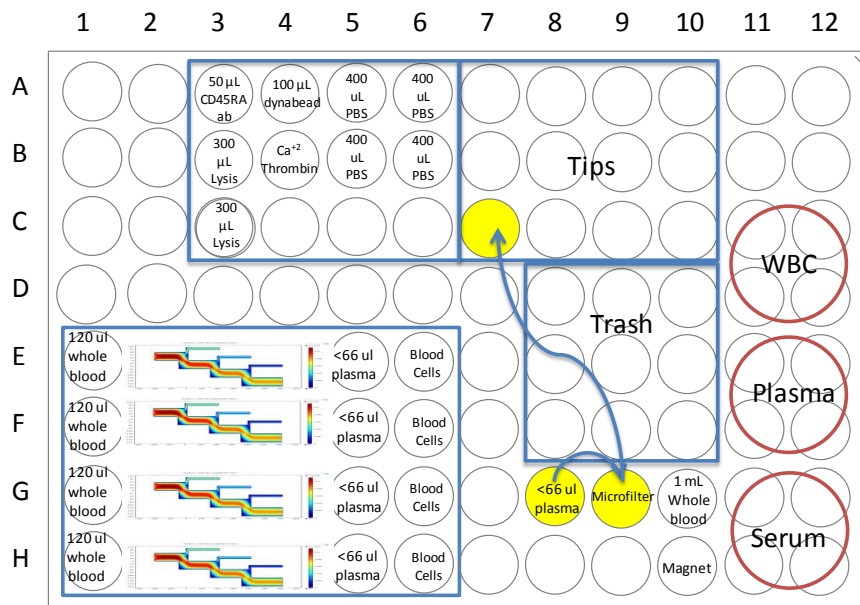
Step 4: Pick up tip, add 10 µL .05mg/mL thrombin and 10 µL 3.5 M calcium. Step information: (t = 0:06, tips = 1). Cumulative information: (t = 20:36, tips = 3)



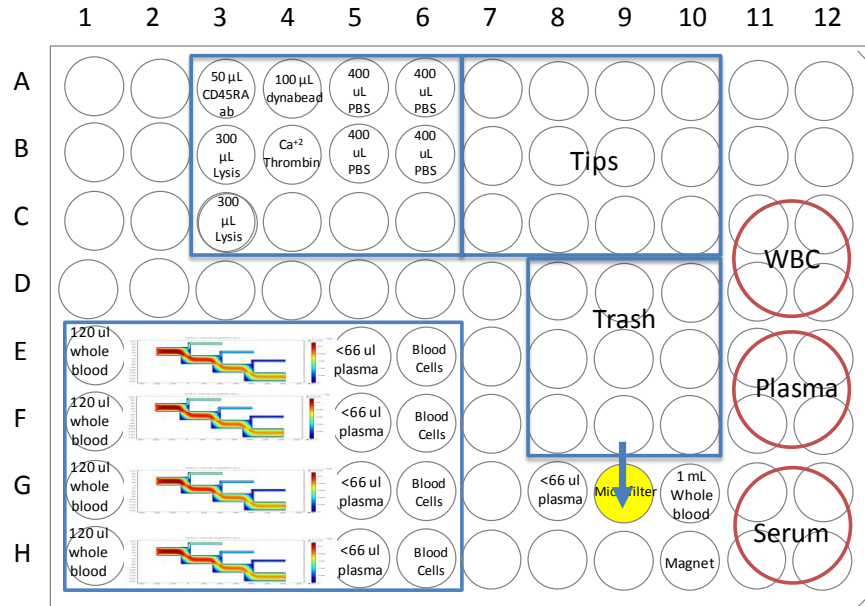
Step 5: Use tip to gently mix. Step information: (t = 0:10, tips = 0). Cumulative information (t = 20:46, tips = 3)



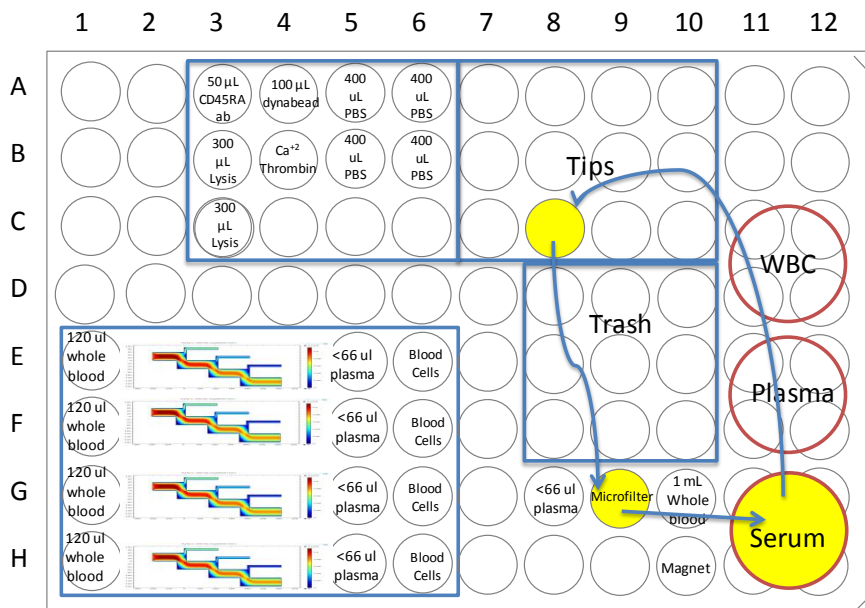
Step 6: Transfer solution to well with microfilter, return tip. Step information (t = 0:06, tips = 0). Cumulative information (t = 20:52, tips = 3)



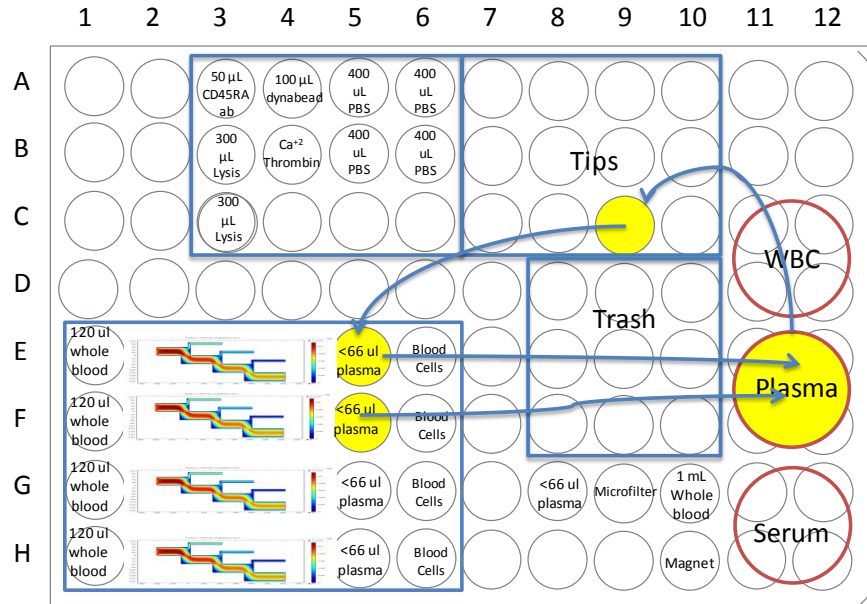
Step 7: Use liquid handler to push solution through filter. Step information (t = 0:06, tips = 0). Cumulative information (t = 20:58, tips = 3)



Step 8: Pick up tip, transfer serum to serum product well, return tip. Step information: (t = 0:09, tips = 1). Cumulative information (t = 21:07, tips = 4).



Step 9: Pick up tip, transfer plasma to plasma product well, return tip. Step information (t = 0:09, tips = 1). Cumulative information



4.1.5 Process Scheduling

In addition to a Gantt chart for the on-cartridge processing steps shown in Figure 4.2, Tables 4.1 and 4.2 display the exact timing, number of wells/tubes and tips involved in each separation unit's steps. This timing was calculated based on channel dimensions as well as the volumes of reagents needed to try and design a cartridge that had a total processing time of under 30 minutes. It should be noted that the timing displayed in the tables is the time required by each of the separation units and not the actual cumulative time since the start of cartridge operation. The Gantt chart displays the real time sequential scheduling of when the liquid handling unit is occupied by each of the separation processes.

The liquid handling unit pauses during the microfluidic plasma separation, allowing it to be used to perform mixing steps in the white blood cell processing sequence. Experimentally, we observed that such starting and stopping of the applied pressure to the microfluidic device does not interrupt flow through the channels for gaps smaller than approximately 1 minute. This advantageous feature of the microfluidic chip allows parallel processing to occur on the cartridge, expediting the total amount of time required to generate the three outputs.

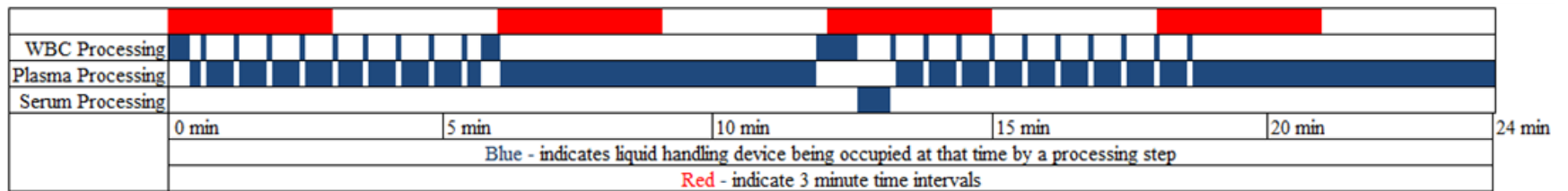


Figure 4.2: Gantt Chart Displaying Liquid Handler Scheduling During Various Separation Processes

Step	Description	Tube/Step	Tips/Step	Time/step	Cumulative Tubes	Cumulative Tips	Cumulative Time
1	Machine picks up 1st tip	0	1	0:02	0	1	0:02
2	Draw 1 mL whole blood	0	0	0:02	0	1	0:04
3	Pipette 1 mL whole blood into 1st well, put tip back	1	0	0:05	1	1	0:09
4	Machine picks up 2nd tip, withdraws 25 uL antibody	0	1	0:04	1	2	0:13
5	Machine pipettes ab into whole blood, picks up 75 uL beads and pipettes into blood	0	0	0:06	1	2	0:19
6	Mix 25 s with tip, put tip back, use hand for other process, come back with same tip, repeat 5 min	0	0	5:00	1	2	5:19
7	Transfer bead, ab, blood to magnetic well using same tip, let magnet concentrate	1	0	2:00	2	2	7:19
8	Machine picks up 3rd tip	0	1	0:03	2	3	7:22
9	Machine withdraws 0.7 mL supernatant and disposes in waste	0	0	0:06	2	3	7:28
10	Machine puts 3rd tip back, picks up 4th	0	1	0:05	2	4	7:33
11	Machine withdraws 0.7 mL PBS	0	0	0:02	2	4	7:35
12	Gently pipette up and down, move liquid (0.7 mL) to trash, put 4th tip back	0	0	0:10	2	4	7:45
13	Machine picks up 5th tip, withdraws 0.7 mL PBS	0	1	0:05	2	5	7:50
14	Gently pipette up and down, move liquid (0.7 mL) to trash, put 5th tip back	0	0	0:10	2	5	8:00
15	Machine picks up 6th tip, withdraws 400 uL 1X lysis buffer	0	1	0:05	2	6	8:05
16	Add buffer to cells and pipette mix 30 s on/off for 5 min	0	0	5:00	2	6	13:05
17	Transfer cell lysate to WBC product well	1	0	0:05	3	6	13:10

Table 4.1: White Blood Cell Processing Steps

<u>Step</u>	<u>Description</u>	<u>Tube/Step</u>	<u>Tips/Step</u>	<u>Time/step</u>	<u>Cumulative Tubes</u>	<u>Cumulative Tips</u>	<u>Cumulative Time</u>
1	Pick up tip, transfer 120 ul of plasma into 4 wells on microfluidic chip, and put tip back	4	1	0:18	4	1	0:18
2	Bifurcation Process	0	0	20:00	4	1	20:18
3	Pick up tip, transfer 200 μ L plasma into another well for serum separation process, return tip	3	1	0:12	7	2	20:30
4	Pick up tip, add 10 μ L thrombin and 10 μ L calcium	0	1	0:06	7	3	20:36
5	Let reagents sit in plasma	0	0	1:40	7	3	22:16
6	Transfer soln. to well with microfilter, return tip	1	0	0:06	8	3	22:22
7	Let reagents/plasma sit in filter	0	0	1:40	8	3	24:02
8	Use liquid handler to push solution through filter	0	0	0:06	8	3	24:08
9	Pick up tip, transfer serum to serum product well, return tip	1	1	0:09	9	4	24:17
10	Pick up tip, transfer plasma to plasma product well, return tip	1	1	0:09	10	5	24:28

Table 4.2: Plasma and Serum Processing Steps

4.1.6 Flow through the Microfluidic Chip

To determine the optimal microfluidic design for our plasma separation, six different geometries were designed in the 2-D CAD software Solidworks and are shown in Figure 4.3 below. Each of the six geometries was tested in COMSOL to ensure that they met the required 4:1 flow rate ratio as described in Chapter 6. For simplicity, only one bifurcation channel was modeled, although one design incorporated three bifurcation channels. After experimental testing, design 2 was selected as the optimal design, with a main channel width of 60 μm and a daughter channel width of 15 μm and a constant depth of 35 μm .

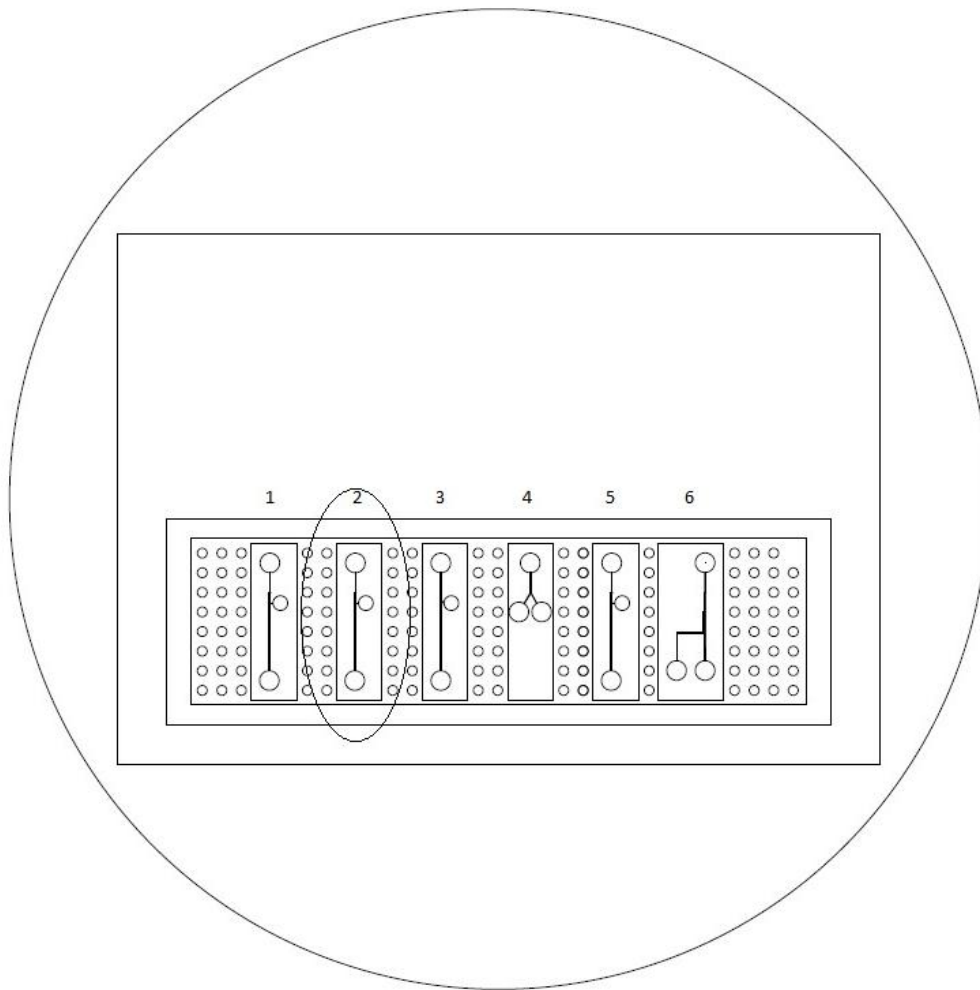


Figure 4.3: Six microfluidic designs constructed in 2-D Solidworks.

4.1.7 Process Conditions and Reagent Volume

All reagents should be stored at -80 °C until the cartridge is in use. This measure is intended to preserve biological reagents such as thrombin and the antibodies. Reagents such as the calcium and lysis buffer are stable indefinitely at room temperature, but for convenience, may be stored in the cartridge insert with the other reagents at -80 °C.

Thrombin must be rehydrated from its powdered stored form in TBS to obtain a final concentration of 0.05 mg thrombin/mL. This should be done by the user immediately prior to use of the cartridge.

The process occurs at room temperature with flow rates as specified elsewhere in and according to the scheduling presented in this chapter. Reagent volumes are defined on the cartridge layout.

4.2 Microfluidic Channel Design

4.2.1 Introduction to COMSOL

COMSOL Multiphysics is a finite element analysis package that can be used to simulate various physics and engineering problems. It has various physics models, namely one for creep flow ($N_{Re} \ll 1$) that we used for optimizing our design prior to fabricating a prototype.

4.2.2 Design Strategy Using COMSOL

Objective

Based on the principles discussed in Chapter 6, we used COMSOL's geometry tools to design a main channel that split into three daughter channels as shown in Figure

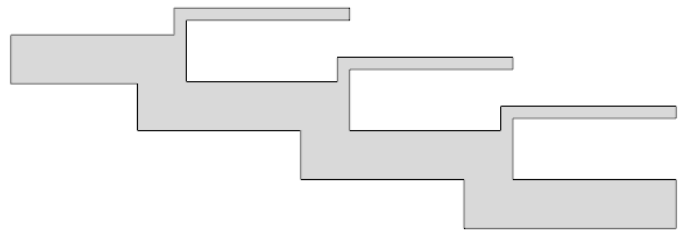


Figure 4.4: Microfluidic design geometry.

4.4. Our objective was to design a system in which the ratio of the flow rates through the smaller daughter channels to the flow rates through the larger main channel was at least 4.0. As discussed in Chapter 6, this situation has been shown to be optimal for nearly pure separation of plasma from the other blood components (3).

We were able to approximate the design as two dimensional using COMSOL's shallow channel approximation. Due to manufacturing limitations, we were able to design a channel with a minimum thickness of 45 μm .

Boundary Conditions & Other Specifications

COMSOL was used to determine steady state operation of the device. An inlet condition corresponding to the inlet on the left hand side of Figure 4.4 was set to 10.5 $\mu\text{L}/\text{min}$. The four outlets on the right hand side of Figure 4.4 were set to an outlet pressure of 1 atm. The material

through the entire device was approximated as water, although blood is known to have a slightly higher viscosity (~5 – 10 cP) than that of water (~1 cP). This assumption was justified since plasma is 93% water by volume and the ratio of viscosities of blood and water is under ten. Therefore, we assumed this approximation introduced negligible error while allowing us to grossly simplify the simulation. After this, the simulation was run to determine steady state conditions through each section of the microfluidic device.

Analysis of the Results

Results were analyzed primarily to determine the ratio of the flow rate through the daughter channel to the flow rate through the main channel for each bifurcation. To this effect, COMSOL was used to determine the average velocity through the daughter channel and main channel through each bifurcation. The flow rate through a channel is most generally expressed by Equation 4.1, below where Q is the channel's volumetric flow rate, u is the linear velocity through the channel and $A_{\text{cross sectional}}$ is the cross sectional area of the channel.

$$Q = u \cdot A_{\text{cross sectional}} \quad (4.1)$$

Furthermore, the cross sectional area of a rectangular channel is given by Equation 4.2 where H_{channel} is the height of the channel in the y-axis direction in Figure 4.4 and T_{channel} is the thickness of the channel in the z-axis (not shown), which was constant for all parts of the device at 35 μm .

$$A_{\text{cross sectional}} = H_{\text{channel}} \cdot T_{\text{channel}} \quad (4.2)$$

Since the thickness of the channel was constant for the device, the ratio of two flow rates could be expressed by Equation 4.3.

$$\frac{Q_1}{Q_2} = \frac{u_1 H_1}{u_2 H_2} \quad (4.3)$$

This relationship was used to determine the flow rate ratios for a variety of geometries, some of which are displayed for the reader in the subsequent section.

4.2.3 Results of COMSOL Simulations

	Main u [m/s]	Daughter u [m/s]	Ratio
First Bifurcation	0.047	0.069	1.59
Second Bifurcation	0.031	0.039	1.89
Third Bifurcation	0.024	0.018	3.17

Table 4.3: Ratios for a channel thickness of 10 μm , a daughter height of 15 μm and a main width of 35 μm

	Main u [m/s]	Daughter u [m/s]	Ratio
First Bifurcation	0.054	0.079	1.57
Second Bifurcation	0.040	0.048	1.94
Third Bifurcation	0.032	0.023	3.17

Table 4.4: Ratios for a channel thickness of 10 μm , a daughter height of 15 μm and a main width of 50 μm

	Main u [m/s]	Daughter u [m/s]	Ratio
First Bifurcation	0.071	0.043	3.88
Second Bifurcation	0.064	0.028	5.27
Third Bifurcation	0.061	0.015	9.50

Table 4.5: Ratios for a channel thickness of 35 μm , a daughter height of 15 μm and a main width of 60 μm

	Main u [m/s]	Daughter u [m/s]	Ratio
First Bifurcation	0.064	0.053	2.84
Second Bifurcation	0.053	0.034	3.69
Third Bifurcation	0.048	0.017	6.53

Table 4.6: Ratios for a channel thickness of 35 μm , a daughter height of 20 μm and a main width of 60 μm

For a channel thickness of 35 μm and for a main channel height of 60 μm and daughter channel height of 15 μm (the design we actually implemented), Figure 4.5 below graphically depicts the flow pattern

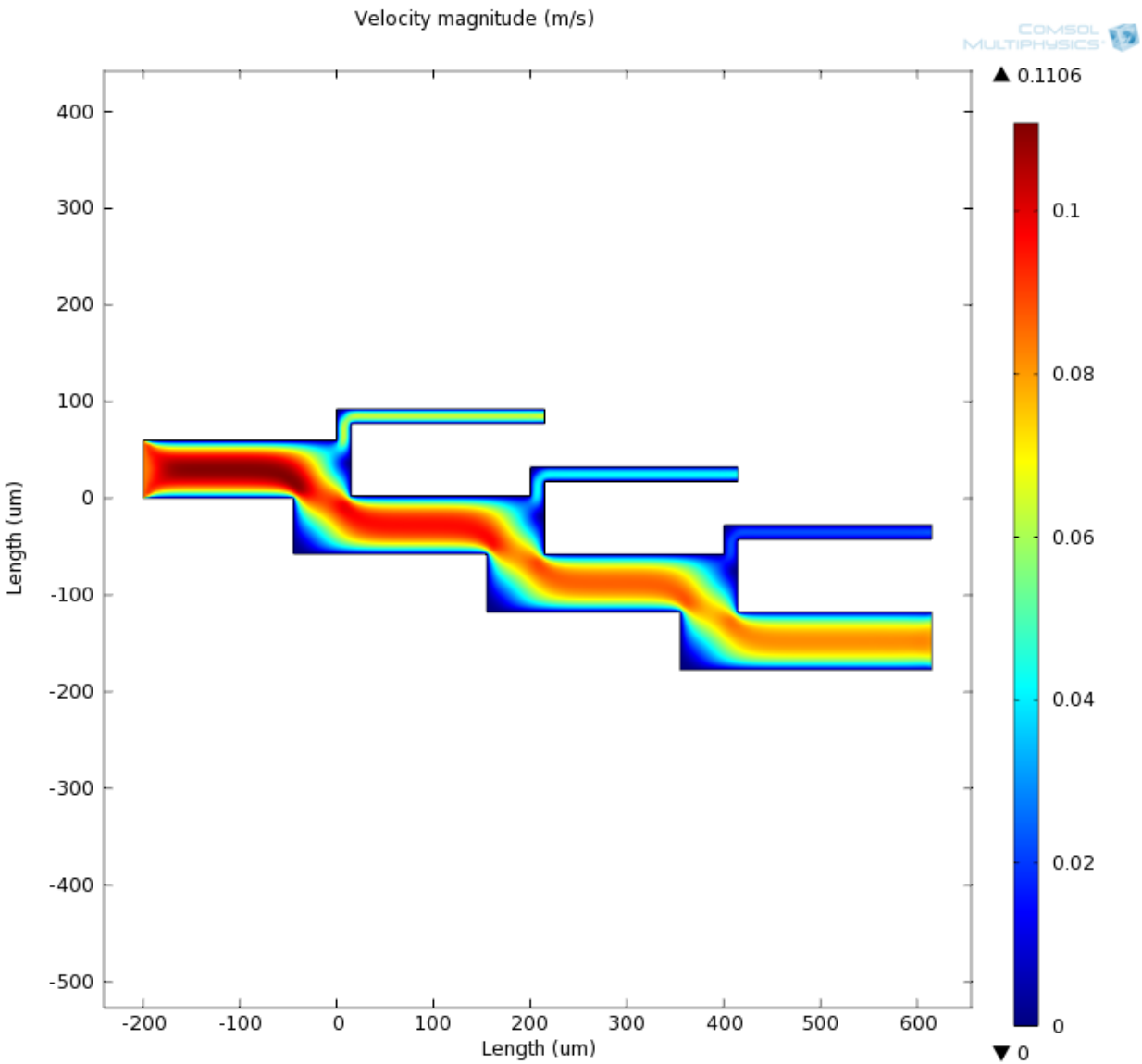


Figure 4.5: COMSOL velocity profile for microfluidic design geometry.

4.3 Serum Filter Design

We considered several designs for the filter unit. Initially, a design in which the filter is internal to the pipette was favored. However, we decided that keeping the filter on the actual cartridge fit our needs best. The design of the filter seen in Figure 4.6 is based off of a standard U-bottom 96 well plate. The membrane and backing is located at the bottom of the well where

the U-bottom begins. The walls of the plate are slightly narrower here to provide extra support to the membrane. At the top of the well is a simple rubber septum which has been punctured to allow the pipette to enter the well. This will provide a seal as the pipette enters the well. This

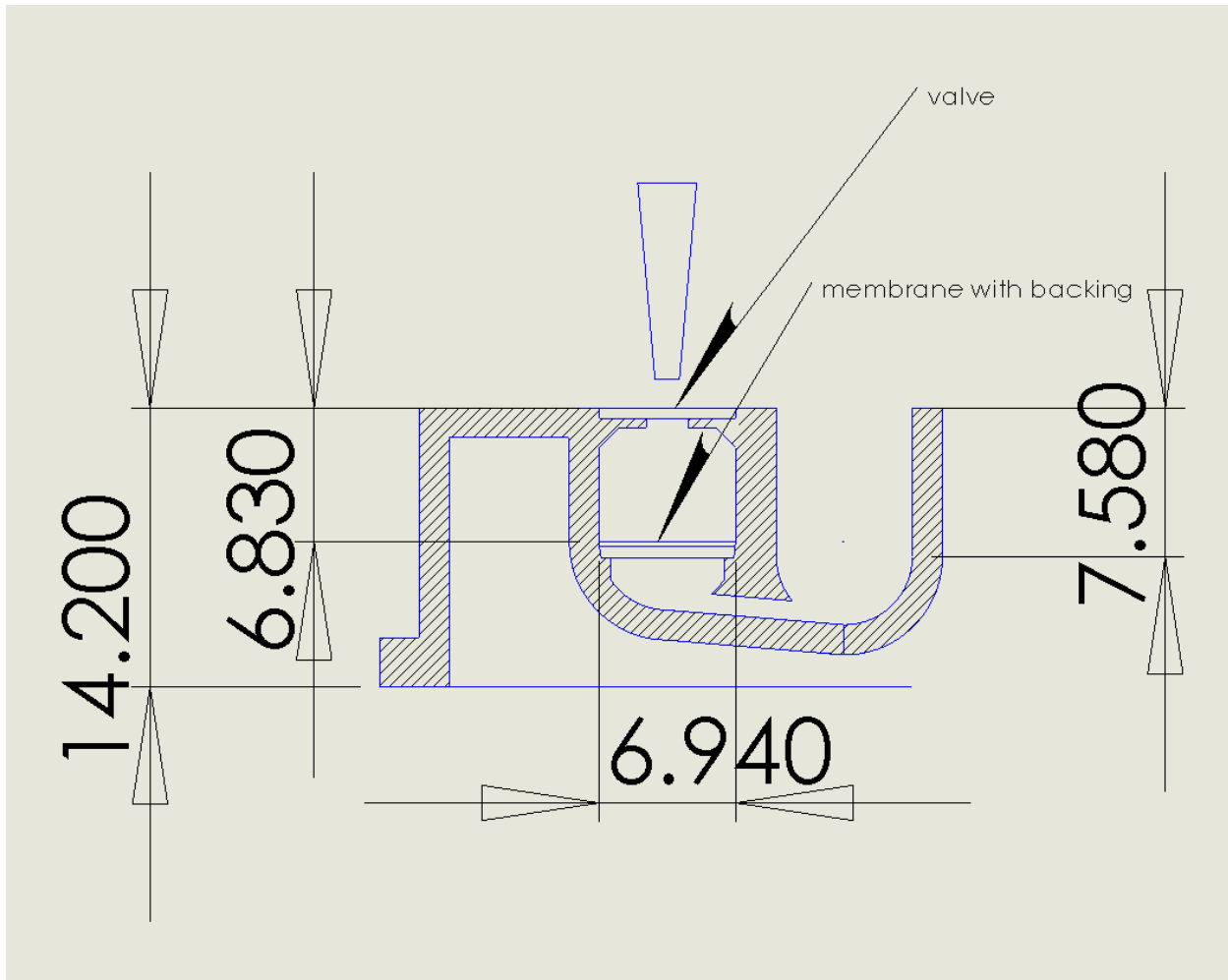


Figure 4.6: Cross section of the filter. All numbers have units of mm

also has a plastic backing to provide additional support. The filtered serum collects in a neighboring hood.

The filtering process begins with the transfer of plasma with clotting factors into the well before the gel point has been reached. After this has fully clotted, the pipette again enters the well and pushes at 50 psi for 5 seconds. As the serum is pushed through the filter, it will flow

into the neighboring well, which is positioned slightly below the filter well to reduce holdup.

With a new tip, the fluid handler withdraws the serum from the neighboring well and transfers it to the final serum container.

The scheduling of the serum filtration is derived from the kinetics of the clotting reaction.

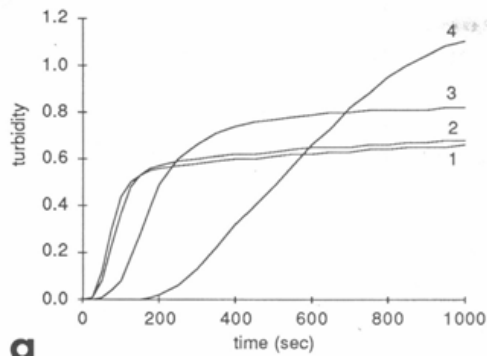


Figure 4.7: Turbidity indicating the progression of clot formation at thrombin concentrations of 1) 1 NIH unit/mL 2) .01unit/mL 3) 0.01 unit/mL and 4) 0.001 unit/mL. The rates of 1 and 2 are nearly identical, with 3 and 4 being significantly slower

Luckily the kinetics of this complex series of reactions has been studied extensively (7). Multiple species are created in multiple reactions, the rates of which impact the overall structure and properties of the resulting clot.

Although multiple factors have an effect on the rate of this reaction, the two most important for us are the fibrinogen and thrombin concentration. Thrombin concentration has an effect on the rate of coagulation, up to a maximum concentration of 0.1 NIH unit/mL (an NIH unit is an

arbitrary measure of catalytic activity of an enzyme). At this point, an increase in the concentration of thrombin does not appreciably accelerate the rate of reaction (Figure 4.7). Thus we chose to use a concentration of 1 NIH unit/mL thrombin. In addition, CaCl_2 is added, as it is essential for thrombin to function properly.

In our separation we hope to both have a complete clot as quickly as possible, and to consume all of the fibrinogen and protofibrils. As shown in Figure 4.8, this is accomplished by letting the reaction proceed for about 225 seconds. We broke this into two sections for two reasons. First, since fibrinogen and protofibrils are relatively small species, allowing the plasma to sit in the filter for the full 225 seconds before filtering could allow some of the fibrinogen and protofibrils to filter through with gravity as the driving force. By transferring to the filter after

100 seconds of clotting, all of the fibrinogen and most of the protofibrils will be consumed, but the plasma will not have reached the gel phase yet. Second, this transfer step will mix the plasma, which will hopefully speed the reaction.

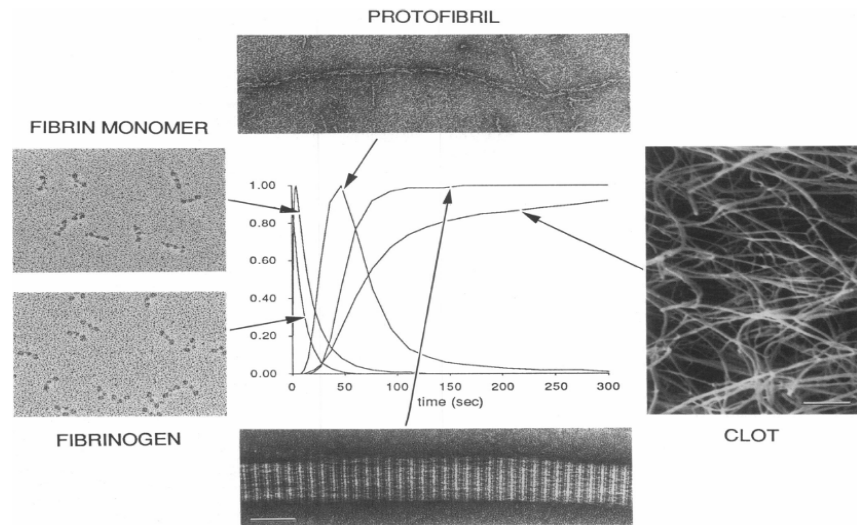


Figure 4.8

4.4 Experimental Data from Prototype Microfluidic Device

4.4.1: Experimental Design and Purpose

A prototype of the microfluidic chip of our cartridge was built in a manner similar to the soft photolithography previously described. A photomask was designed in Solidworks and printed in high resolution (10,000 DPI). Then, a negative photoresist was spun onto a silicon wafer at 4000 RPM for 30 seconds to achieve a thickness of 45 μm . Following a soft bake at 95 $^{\circ}\text{C}$ for 15 minutes, the wafer was aligned with the printed photomask in a Karl Suss mask aligner and exposed to 365 nm UV light to achieve 975 mJ/cm^2 of exposure energy. Afterwards, a post exposure bake occurred at 95 $^{\circ}\text{C}$ for 3 minutes and all un-reacted photoresist was removed with a developer for 10 minutes under frequent

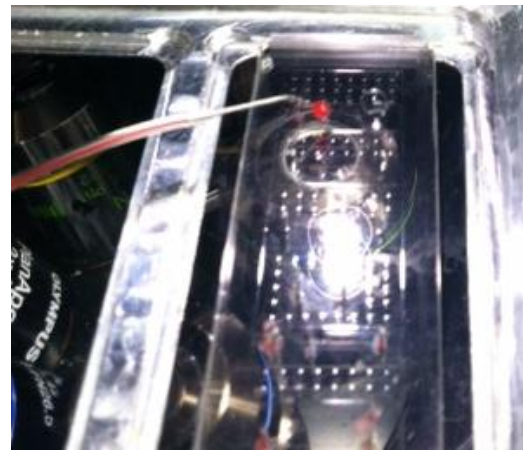


Figure 4.9: Fabricated PDMS microfluidic chip attached to a glass slide. As shown here, a clear plasma fraction (right) and concentrated blood output (red droplet, left) can be observed.

agitation. Then, in a mixture of 1:10 mixture of PDMS and curing agent was poured into the mask and placed into an 80 °C oven for 2 hours. After this step, the device was removed, cut, sealed to a glass slide, and ports were punched to allow for outlet collection and pressure input.

The main intention of this testing was for proof of concept purposes. The chip included six total designs with the same channel dimensions, but different types of bifurcations (as shown in Figure 4.9). All of these designs had a main channel width of 60 μm , a daughter channel width of 15 μm and a depth of 45 μm . The same flow rates and fabrication process was used as would have been for the complete device shown in previous figures. Therefore, testing was intended to demonstrate three things: 1) that the chip design was capable of being manufactured, 2) that the microfluidic principles underlying the design were effective at separating blood and 3) that the flowrates being used would be sufficient without either destroying the device or causing shear of the blood cells.

It should be noted that there were a few important differences between the testing and the way the cartridge will work in the liquid handling unit. First, lab equipment was used to substitute functions the liquid handler would normally perform. The liquid handling robotic arm was substituted with a syringe pump from Harvard Apparatus in order to apply a constant flow rate (from 10 $\mu\text{L}/\text{min}$ to 25 $\mu\text{L}/\text{min}$ during testing) across the channels. Blood was collected in a citrated tube from a willing volunteer and then placed in a syringe to be pumped through the device. Flow rates ranging from 10 $\mu\text{L}/\text{min}$ to 25 $\mu\text{L}/\text{min}$ were tested through each of the six designs. The chip was mounted on an inverted microscope so that pictures of the device in action could be obtained. Initial trials using the device without modification and with whole blood showed no plasma separation under any flow rates. However, subsequent trials aimed at increasing the resistance to flow through the plasma channels demonstrated that the device does

indeed fractionate the blood into a plasma fraction and a remaining blood fraction. These results are discussed next.

4.4.2 Preliminary Qualitative Results

The device was first viewed under the microscope to ascertain whether or not the fabrication process maintained structural fidelity to the SolidWorks mask (Figure 4.3) we designed. The figures below examine some of the various designs to demonstrate that, indeed, the channels maintained their structural accuracy through the soft photolithography process. In the figures below, three designs are examined, a 3-channel bifurcation with rounded square edges, an angled bifurcation and a “y” bifurcation (a-c, respectively).

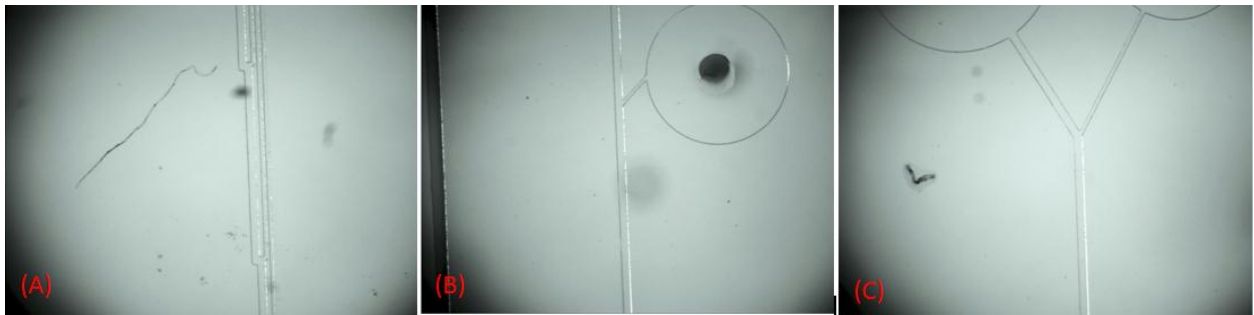


Figure 4.10: A – three bifurcations in series with rounded square edges. B – a single, angled bifurcation pattern. C – a single “y” shaped bifurcation pattern

As mentioned earlier, initial trials of running the device with human whole blood did not work under any flow rates. The blood simply split its flow but stayed as whole blood as it flowed through the channel. No images were taken of this process

Furthermore, it was observed that for flow rates up to 15 $\mu\text{L}/\text{min}$, no blood shearing was observed, indicating that the process could potentially be made faster during year of clinical testing. Under dilution of the whole blood to 10% hematocrit, flow rates up to 25 $\mu\text{L}/\text{min}$ could be achieved.

Finally, preliminary testing showed that removal of the syringe pump pressure did not immediately stop flow, because of the pressure buildup in the channels associated with

microfluidic flow. This observation is critical to correct operation of the device in the cartridge as shown in the Gantt chart, since the liquid handling unit must take breaks from pushing the fluid through the microfluidic chip in order to perform parallel mixing steps.

4.4.3 Troubleshooting and Further Qualitative Results

Taken together with our outputs from the COMSOL simulation, preliminary experimental results suggested that some of the assumptions inherent in our COMSOL model were incorrect, resulting in a flow pattern that was not conducive to plasma skimming. Namely,

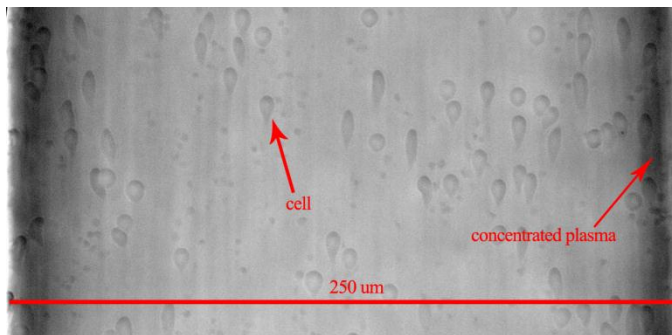


Figure 4.11: Flow through a longer path length channel with a width of 250 μm produced concentrated plasma towards the channel edges near the channel outlet. This phenomenon could prove useful in designing a chip to "skim" plasma from whole blood.

our COMSOL model approximated the material properties of blood as those of water. While the viscosities are similar, since blood is 93% water by volume, this approximation most likely introduced an error into calculating the ratio of resistances through the daughter channels after the bifurcation. Since it is this ratio that

governs how the blood will split into plasma and cell/particulate fractions, any calculation errors manifested physically in a flow pattern that did not cause separation of the blood. Furthermore, the material was assumed to be constant throughout the device domain for the purposes of simplifying the COMSOL simulation. Again, though the viscosities of plasma and the concentrated blood fraction are similar, they are not the same, introducing further error in the resistance calculations. Finally, the COMSOL model did not take into account the total length of the device in calculating resistances and pressure drops. In normal industry applications where turbulent flow is common, the effect of pipe length is usually negligible. However, in

microfluidics, where creep flow is prevalent, the effect of total channel length is likely not negligible.

This suggestion is backed up by the following experimental evidence. We tested an existing microfluidic device with a total path length of almost 10 cm (in comparison to the 1-2 cm path lengths in our prototype). Though the channel width was approximately 250 μm , making direct comparison with our designs difficult, flow was examined at the outlet of the channel. As can be seen in Figure 4.11, plasma seems to concentrate towards the edges of the wall while blood cells migrate towards the center of the flow profile. Future designs could take advantage of this phenomenon through a three way channel split to skim plasma from the remainder of whole blood.

Next, we placed a 100 μL droplet of water at the outlet of the plasma channel on our prototype design with the intention of providing a hydrostatic pressure head to increase resistance to flow through that channel. This was done for a few different bifurcation styles. Assuming a completely spherical drop and a density of 1 g/cm^3 , this droplet corresponded to a pressure head given by Equations (4.1) and (4.5):

$$V = 0.1 \text{ cm}^3 = \left(\frac{4}{3}\right) \pi r^3 \rightarrow r = 2.88 \times 10^{-3} \text{ m} \quad (4.4)$$

$$P = \rho * g * r = .056 \text{ Pa} \quad (4.5)$$

This additional pressure head was sufficient to effect separation of plasma from whole blood as shown in Figure 4.12 below.

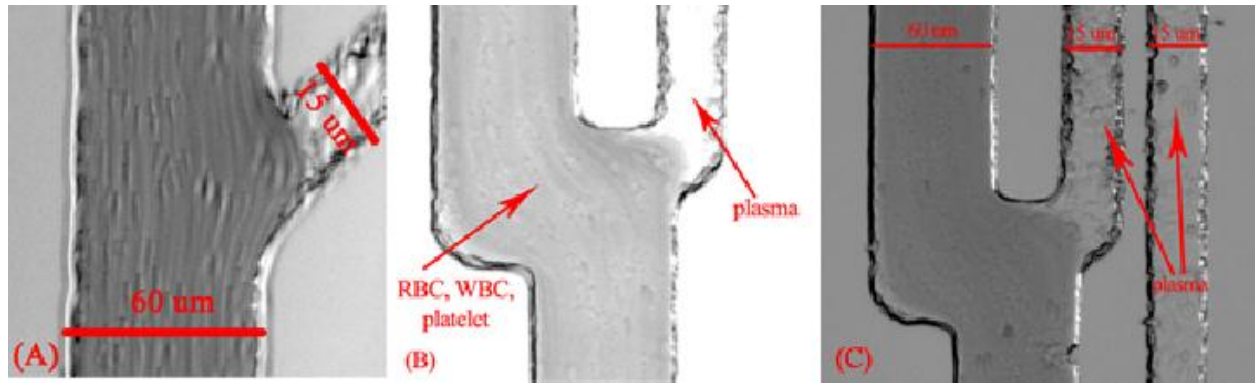


Figure 4.12: A - angled bifurcation working under increased hydrostatic pressure on plasma channel. B - rounded square bifurcation working under increased hydrostatic pressure. C - rounded bifurcation in final design working under increased hydrostatic pressure

The results of this set of experiments fairly conclusively demonstrate that indeed, the bifurcation principle should hold. Unfortunately, the simplifying assumptions in our COMSOL model did not allow for the correct calculations of the resistance ratios. However, knowing this *a priori* would have been fairly difficult, and with empirical data to help uncover this flaw, the model can be updated and the design improved during the clinical trial year.

Blood smears were taken for the outputs of both plasma and whole blood to determine the purity of the plasma fraction obtained. As can be seen in Figure 4.13, the plasma output (A) has considerably fewer blood cells than the blood output (B) from the channel. Qualitatively, this demonstrates that the technique is suitable for use in separating plasma from whole blood.

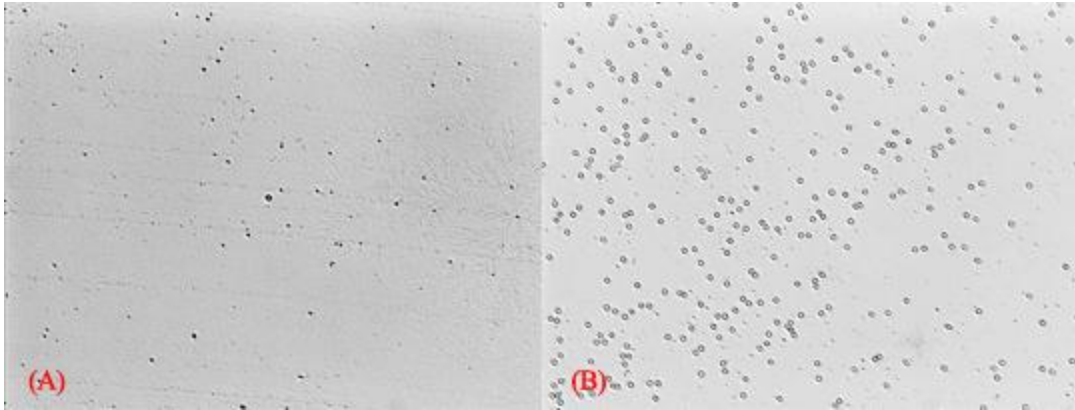


Figure 1: A - plasma output from device. A nearly pure plasma fraction (free of cells and other blood debris) is obtained. B - blood output from device contains considerably more cells and other debris from whole blood

Thus, all experimental data adequately supports the aforementioned proof of concept points. With minor modifications based upon the results of the experiments, the device should be able to be produced in a manner that successfully accomplishes the point of the product while maintaining the size and costs of the device mentioned here in the report.

Chapter 5: Blood Composition

5.1 Introduction

Germane to understanding the point of the Diamond HemeSep is a working knowledge of the biology and composition of human whole blood. To provide such a basis, this chapter presents a very simple introduction to the topic.

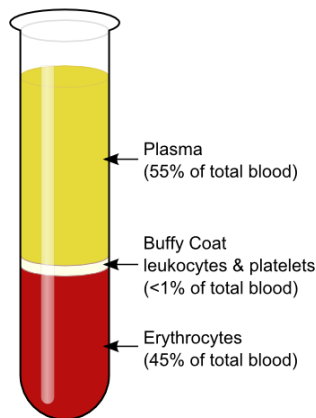


Figure 5.1: Human whole blood is composed of three fractions: plasma, white blood cells (leukocytes) and red blood cells (erythrocytes) (8)

Human whole blood is an important bodily fluid that delivers oxygen and nutrients while removing metabolic waste from the cells and tissues of the body. Blood is composed of three primary constituents as shown in Figure 5.1: 1) red blood cells (erythrocytes), 2) white blood cells (leukocytes) suspended in a liquid 3) plasma. Hematocrit, which is defined as the percentage of red blood cells by volume in whole blood, is typically around 45% in humans (8). The three components of blood will be discussed next.

5.2 Plasma

Human blood cells are suspended in a straw-colored liquid component of blood known as plasma. Plasma, which is mostly water (93% by volume), also contains dissolved chemical species. Primarily, plasma transports glucose, clotting factors, minerals, ions, hormones, carbon dioxide, and proteins such as albumin. As such, plasma serves as a major protein reserve in the body and also as the major medium for waste removal. Plasma constitutes around 55% of whole blood by volume (8, 9).

Because of its crucial role in regulating blood osmolarity and infection prevention, plasma is often analyzed for its salt and protein concentrations both clinically and in research. Consequently, obtaining nearly pure plasma fractions is very important.

5.3 Serum

Blood serum is essentially blood plasma with fibrinogens (proteins used in the clotting process) removed. Serum does not contain any blood cells, but does contain all of the proteins not used in the clotting process and otherwise found in the plasma. Like blood plasma, serum is used in many diagnostic tests both clinically and in research (10).

5.4 White Blood Cells

White blood cells (WBCs), or leukocytes, are the DNA-containing blood cells that make up the body's immune system. These cells protect the body from infectious disease and foreign agents while also modulating inflammatory responses. Five different types of WBCs exist: neutrophils, eosinophils, basophils, lymphocytes and monocytes. The first three are classified as granulocytes due to their granular appearance under the microscope caused by membrane bound enzymes that engulf and destroy foreign matter. The latter two are characterized as agranular due to the absence of granular appearance under the microscope (9, 11).

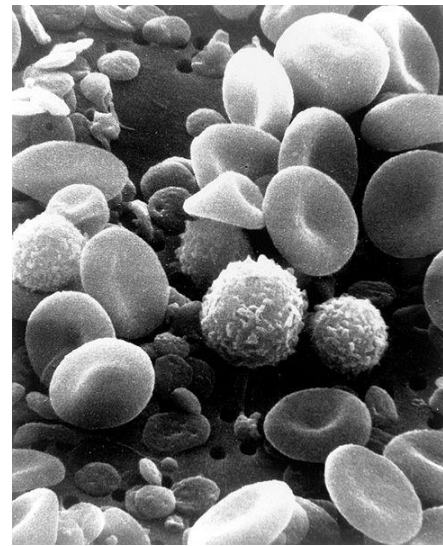


Figure 5.2: WBCs are the irregularly shaped spherical cells whereas RBCs cells are the larger, donut shaped cells. (9)

Analysis of white blood cells is useful and necessary in understanding the DNA profile of a patient and in looking for various markers of disease and health.

5.5 Red Blood Cells

Red blood cells (RBCs), or erythrocytes, are the most common type of blood cell. RBCs deliver oxygen (O_2) to the body's cells and tissues. These cells owe their red appearance and ability to transport O_2 to hemoglobin, an iron containing biomolecule. These cells develop in the bone marrow and circulate for about 100-200 days before being recycled and regenerated. RBCs are anucleate, meaning they do not contain a nucleus and, consequently, do not contain DNA. Per microliter of whole blood, there are roughly $4-6 \times 10^6$ RBCs.

RBCs are generated through a process known as erythropoiesis. They are produced in the bone marrow at a rate of about 2 million cells per second and mature over seven days (12, 13).

5.6 Blood Coagulation

Blood coagulation is the process to form a clot to quickly prevent excessive blood loss after injury. While a quick response is essential, the process must not make clots that impede normal blood flow. Thus the process is highly regulated by multiple positive and negative feedback loops.

There are two pathways in the coagulation cascade (14). Most important *in vivo* is the extrinsic pathway in which injury pushes tissue factor from surrounding subendothelial cells into the blood flow, initiating the cascade. The intrinsic pathway, however, is much more important for our purposes. This pathway is activated by an anionic surface such as glass

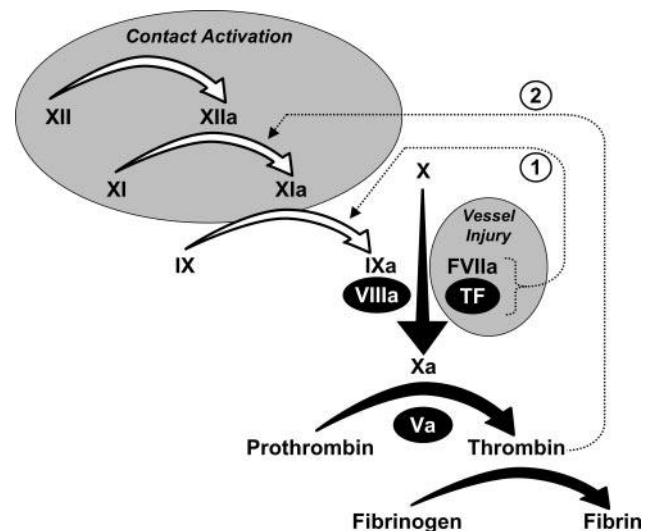


Figure 5.3: Plasma coagulation. Roman numerals indicate unactivated coagulation factors, and activated factors are indicated by a lower case "a". Nonenzymatic cofactors are indicated by numerals in black ovals. White arrows indicate reactions of the intrinsic pathway

(15). Contact with an anionic surface produces the active serine protease XIIa from the inactive form XII. This protease then selectively hydrolyses a portion of the inactive serine protease factor XI to form the active protease XIa. This in turn activates factor IX in the same way, which then activates thrombin from prothrombin in the same way. While the rest of the enzyme factors are involved primarily in regulating coagulation, thrombin is the serine protease actually responsible for the formation of the clot. It is important to note that many of these factors, and specifically the interaction of XII with an anionic surface, depend on the presence of calcium ions. Removal of calcium ions with a chelator such as EDTA or citrate prevents the coagulation cascade.

Essential in any discussion of blood coagulation is the final product: a blood clot or thrombus. There are two main components of any blood clot. First is an insoluble fibrin polymer, produced from the soluble protein monomer fibrinogen. An electron micrograph of such a clot can be seen in Figure 5.4 (7). This polymer can vary in its properties depending on the degree of branching and the thickness of the individual fibers. The second component is blood platelets. Platelets are cell fragments about a quarter of the size of red blood cells. These become ensnared in the network of fibrin and help plug the flow of blood. Finally, small amounts of the other clotting factors become stuck in the clot as well. This helps to down-regulate the coagulation response and limit it to the site of the

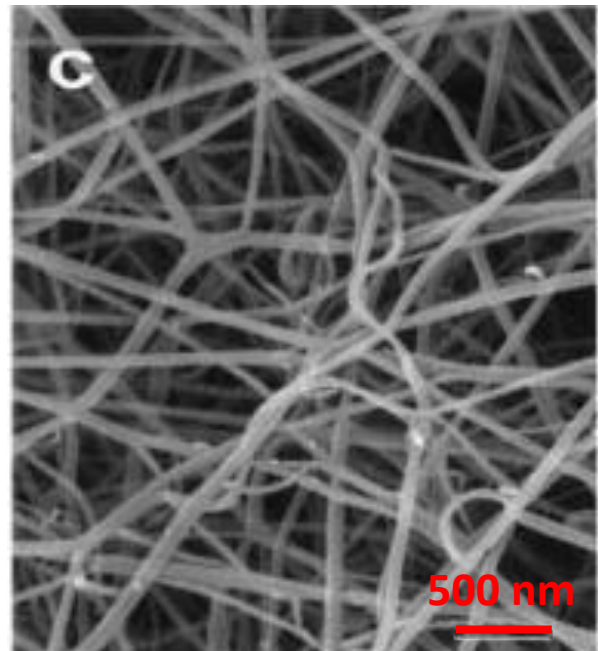


Figure 5.4: Electron micrograph of a blood clot with bar indicating 500nm

injury. By removing these clotting factors, the production of thrombin and then fibrin monomer cease.

5.7 Fibrin Polymerization

The most important process in the coagulation cascade for our purposes is the polymerization of fibrinogen to form fibrin. This is a complex process catalyzed by the serine protease thrombin. Fibrinogen circulates as a dimer of three chains ($A\alpha B\beta\gamma$)₂ which are connected by a disulfide bond. Figure 5.5b shows a crystal structure of the A chain of the fibrinogen monomer (16). The first step in the formation of the insoluble

fibrin clot is the cleavage of part of the $A\alpha$ chain by thrombin to form fibrin I monomers. The fibrinogen-

thrombin complex is shown in figure 5.5a (17). These monomers then aggregate to form fibrin I protofibrils. Third, thrombin cleaves a portion of the $B\beta$ chain to form fibrin II protofibrils.

Finally, these protofibrils rapidly aggregate to form the insoluble fibrin clot.

5.8 Summary

Human whole blood is composed of three main components: 1) red blood cells, 2) white blood cells and 3) plasma. The cells are suspended in plasma, the major liquid fraction of whole blood that also contains many other dissolved species. Blood serum is simply plasma with the fibrinogens clotted out and separated from the rest of the plasma. Each of these blood fractions

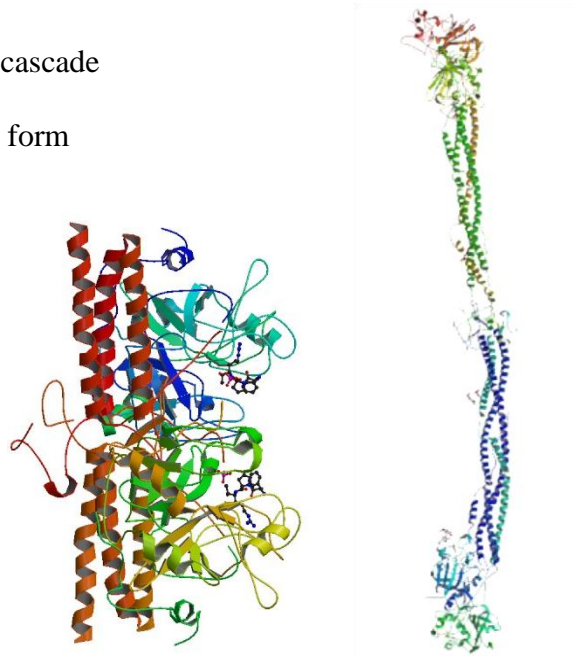


Figure 5.5: a) crystal structure of a portion of fibrin bound to the active site of thrombin. b) crystal structure of fibrin.

is diagnostically useful in its own right, making blood separation an interesting, essential process both clinically and in research.

Chapter 6: Plasma Separation via Microfluidic Design

6.1 Introduction

To induce plasma separation from whole blood without the use of traditional centrifugation techniques, a microfluidic channel design was implemented based on published work by Yang, Undar, and Zahn (3). There are many advantages of utilizing microfluidics over centrifugation, including cost, size, and autonomy of the unit. The efficacy of the device in separating cells from plasma in whole blood stems from the bifurcation law, or the Zweifach-Fung Effect.

6.2 Bifurcation Law (Zweifach-Fung Effect)

The bifurcation law states that when red blood cells encounter a bifurcation in a capillary blood vessel, a large number of cells flow into the daughter vessel that has the higher flow rate, leaving only a small number of cells to flow into the daughter vessel with the lower flow rate. This occurs due to the large pressure gradient of the high-flow vessel compared to the low-flow vessel. Asymmetric shear stresses on the capillary walls also induce a torque on the cell that causes it to flow into the high-flow vessel. It has been shown that a flow ratio of 2.5:1 is sufficient to induce this law in channels whose dimensions are approximately the same as a cell. This preferential bifurcation only occurs, however, when a cell's centroid falls above a critical streamline, the stagnation point of the bifurcation. As the ratio of flow in the daughter vessels is increased, this stagnation point lies closer to the low-flow vessel and effects of this law are more pronounced. Figure 6.1a below diagrams the effects of pressure and shear rate on the bifurcation law while Figure 6.1b describes the critical streamline needed for this preferential bifurcation.

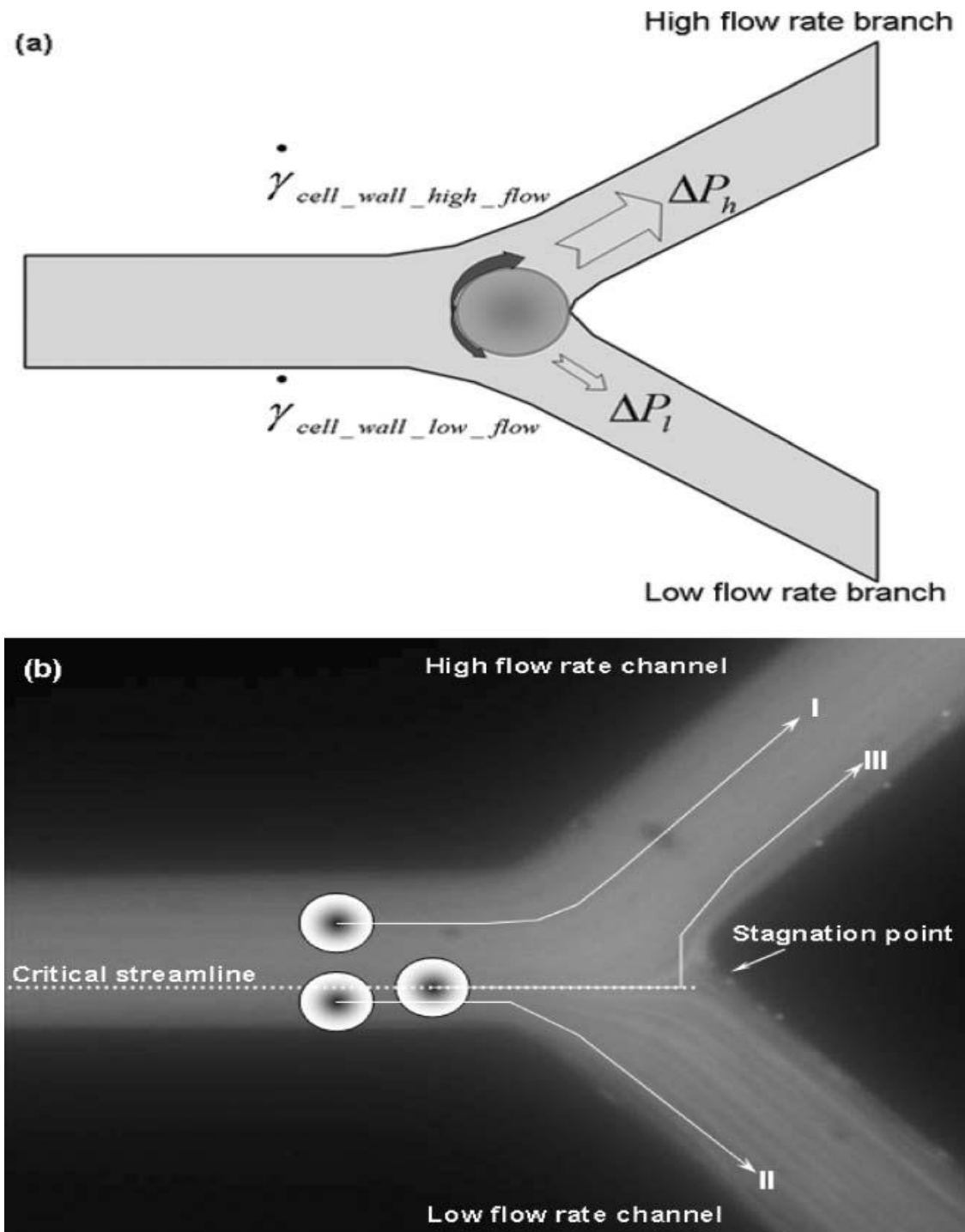


Figure 6.1. Schematic of the bifurcation law (Zweifach-Fung Effect) (3). Figure 1a. shows the effect of pressure drop differences on the cell ($\Delta P_h > \Delta P_l$). The shear rate differences ($\gamma_{cell_wall_high_flow} > \gamma_{cell_wall_low_flow}$) are also presented and are instrumental in producing the Zweifach-Fung Effect. Figure 1b. shows the critical streamline in a bifurcation channel operating at a 4:1 flow rate ratio and the resulting paths of the cells. Path's I and III both flow to the high-flow channel because the centroid of the cell lines above the stagnation streamline. Path II, however, flows into the low-flow channel.

6.3 Flow rate ratio optimization

As mentioned above, the choice of flow rate ratio between the daughter channels is a key design specification in achieving desired separation efficiencies. For use in research applications, the purity of the plasma should be as high as possible without sacrificing its integrity. Yang, Undar, and Zahn show in their research that a 4:1 flow ratio between the channels resulted in a 98.9% recovery efficiency for C8161 Human melanoma cells while ratios above 6:1 resulted in 100% efficiency (3). Their complete analysis is shown in Figure 6.2 below.

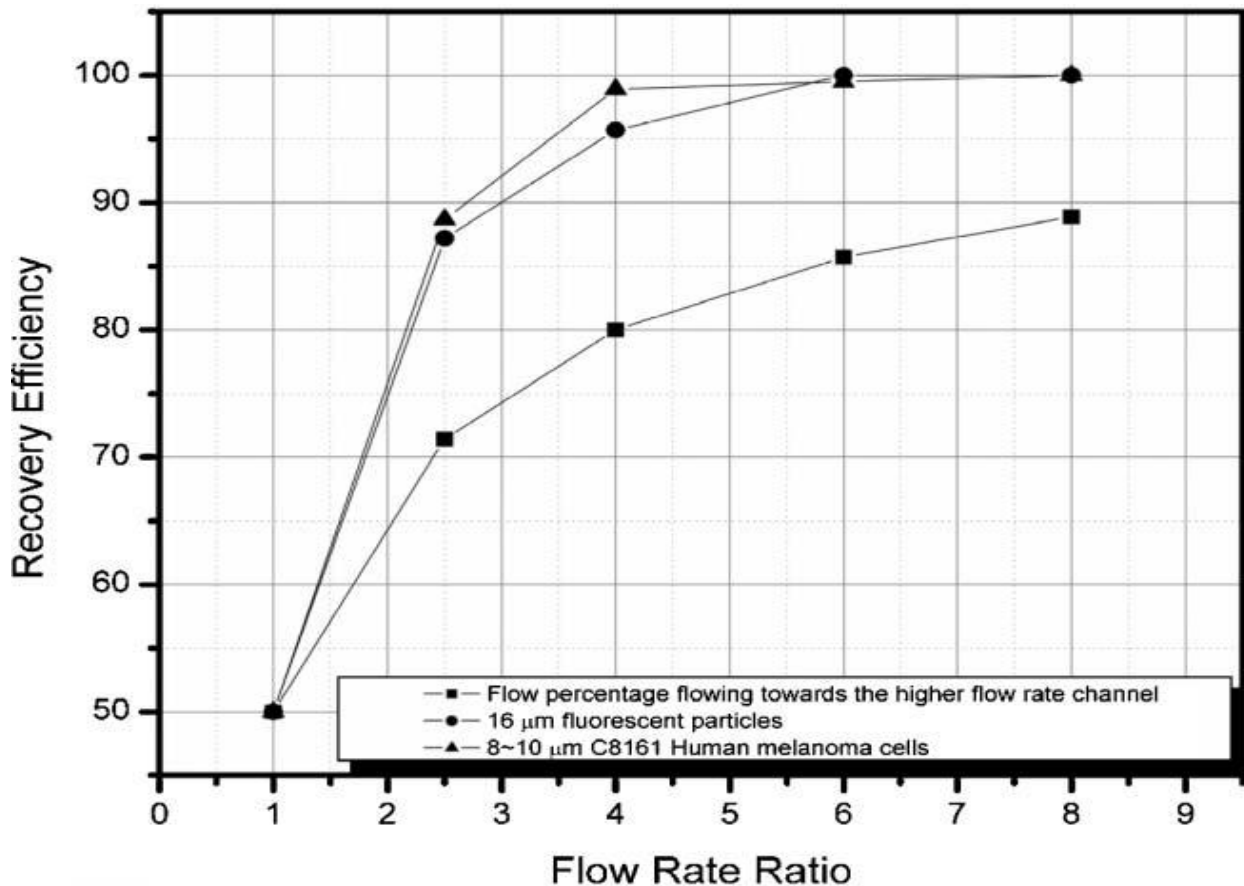


Figure 6.2. Recovery efficiency of 16 μm fluorescent particles and 8-10 μm C8161 Human melanoma cells. It can be seen that at flow ratios greater than 4:1 cell recovery efficiency is virtually 100%. Cell efficiency was consistently greater than the fluorescent particles because the deformity of the cell led to an asymmetric distribution of cells in the middle of the channel, which increased efficiency (3).

6.4 Flow rate resistance and use of multiple parallel bifurcations

To achieve greater plasma yields in the microfluidic device, multiple parallel bifurcations can be incorporated. However, each additional channel causes a drop in flow resistance in the subsequent channels. To prevent efficiency losses in the subsequent channels, each channel diameter must be changed to reflect this. Equation 6.1 below demonstrates the relationship between pressure changes (ΔP), flow rates (Q), and resistance to flow (R):

$$\Delta P = Q \cdot R \quad (6.1)$$

From this equation it is seen that flow resistances will need to be varied to achieve the desired pressure changes. These resistances must therefore satisfy the following equation:

$$R_{p,j} = r_Q \left(1 + \frac{1}{r_Q + 1} R_{p,j-1} \right) R_{B,j} \quad (6.2)$$

Where r_Q is the flow rate ratio, $R_{p,j}$ is the resistance of the j^{th} plasma channel, and $R_{B,j}$ is the resistance of the j^{th} blood cell channel. Yang, Undar, and Zahn performed their analysis on a microfluidic design that incorporated 5 daughter plasma channels. Figure 6.3 below reproduces their velocity profiles and analytical results from using computational fluid dynamics.

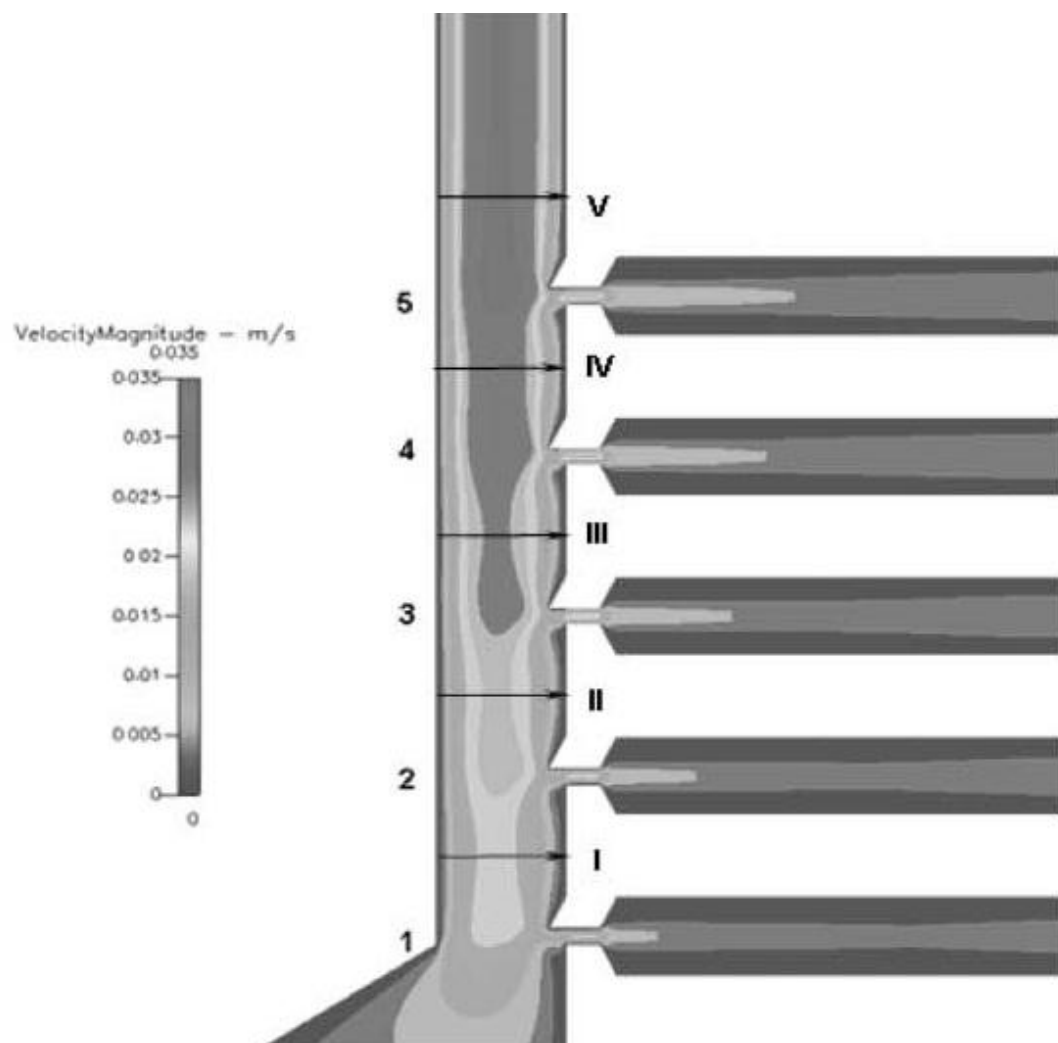
(a)

Figure 6.3a. CFD velocity profile for microfluidic device. Each split results in a decreasing velocity profile.

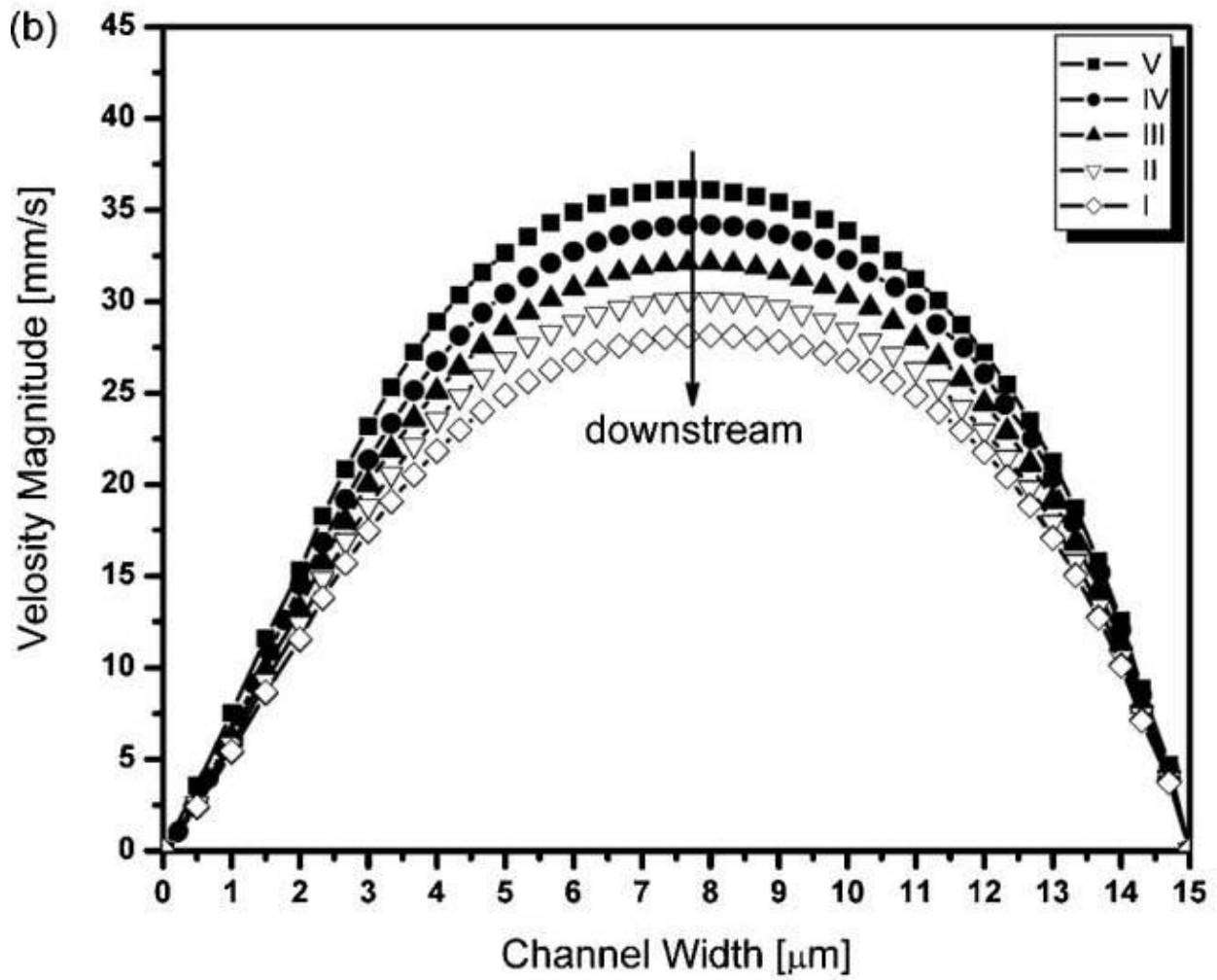


Figure 6.3b. Velocity magnitude at each node for varying channel widths. As blood flows downstream the velocity magnitude decreases proportionally, and the parabolic peak represents the optimal channel width.

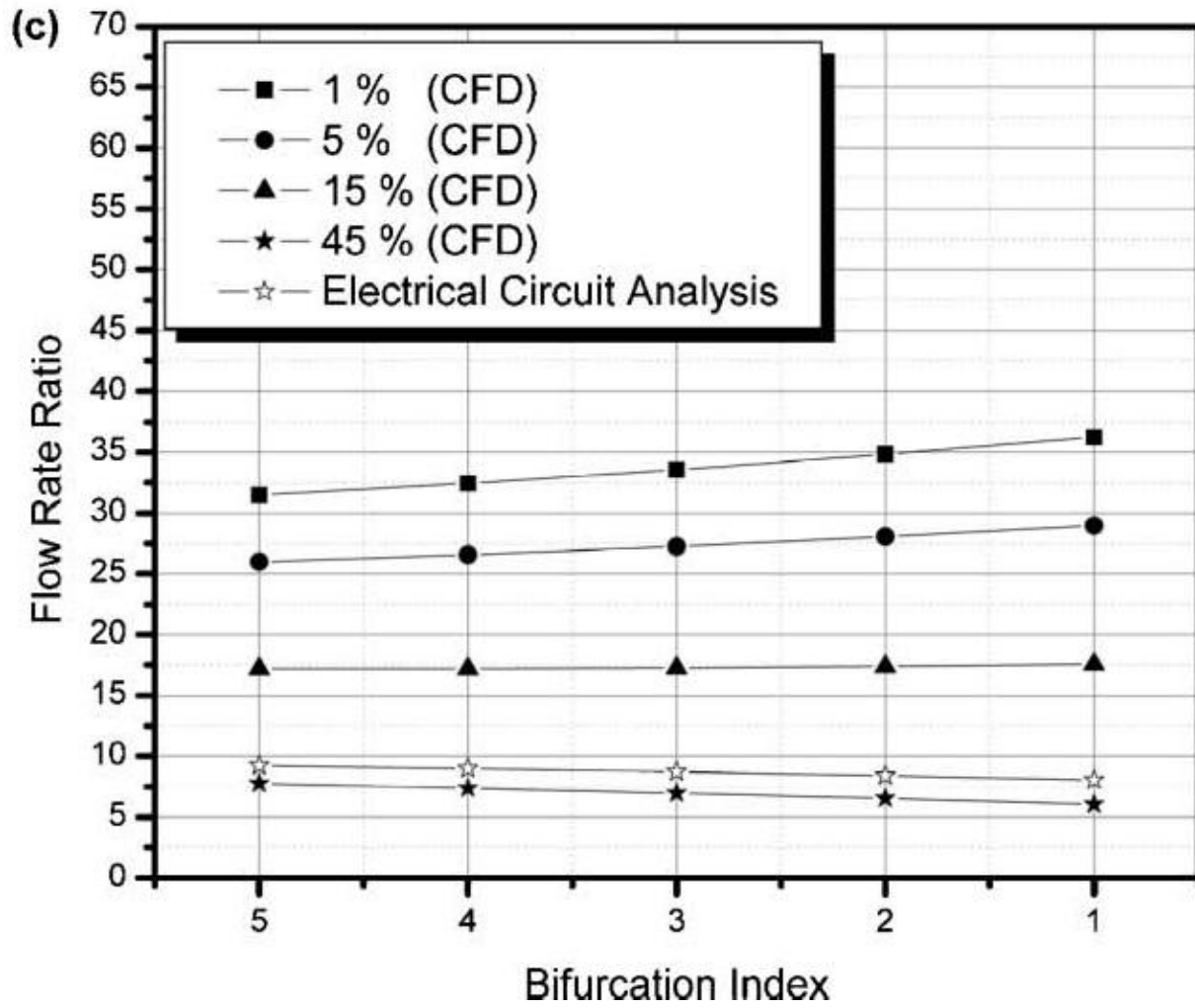


Figure 6.3c. Flow rate ratio for 1%,5%,15,45% hematocrit using CFD analysis. Bifurcation nodes farther downstream result in larger flow rate ratios.

6.5 Overall device efficiency and the Fahreus effect

To test the overall efficiency of the microfluidic device, the percent by volume of plasma separated is quantified. This value (η) can be expressed with the following equation:

$$\eta = 100 \left\{ 1 - \left(\frac{H_{mu}}{H_{md}} - 1 \right) \left(\frac{H_{mu}}{100 - H_{mu}} \right) - \frac{H_{mu}}{H_{md}} \right\} \% \quad (6.3)$$

Where H_{mu} is the hematocrit levels upstream of the separation region and H_{md} is the hematocrit levels downstream of the separation region. When a higher initial inlet hematocrit is used, higher upstream and downstream hematocrit levels are observed. These simultaneous increases lead to higher percent by volume plasma separation, as seen in Equation 6.3. Yang, Undar, and Zahn quantitatively confirmed Equation 6.3, and the results are reproduced in Figure 6.4 below. It has been shown, however, that when a large feed inlet is flown into a channel of smaller diameter, the average hematocrit decreases as channel diameter decreases. This is known as the Fahreus effect and is shown for a 15 μm channel in Figure 6.5 below (3).

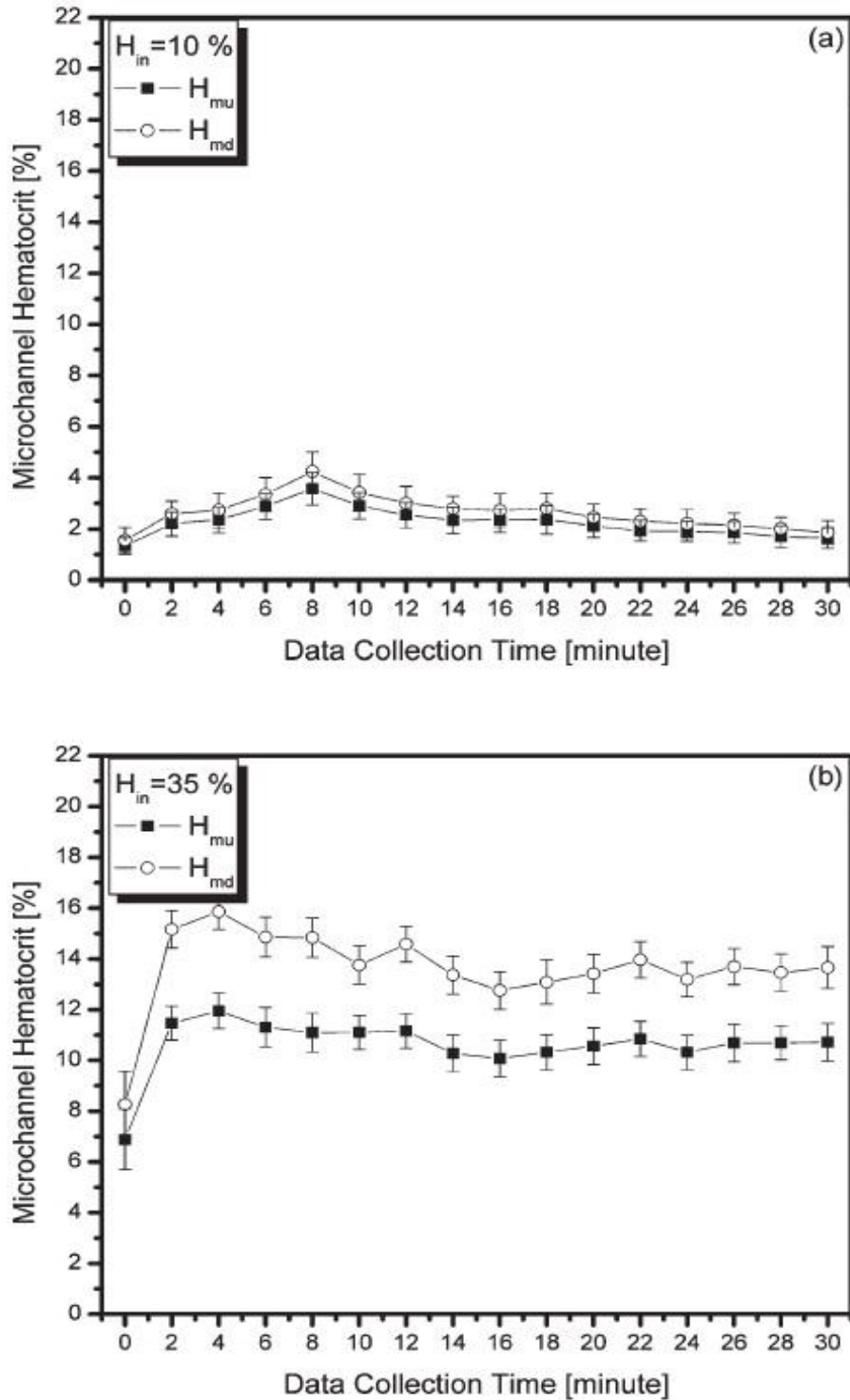


Figure 6.2. Microchannel hematocrit vs. time for inlet hematocrit levels of 10% and 35%. 4(a) represents inlet levels of 10% and 4(b) represents inlet levels of 35%. As inlet hematocrit levels increase, the degree of separation is increased, as expected from Equation 6.3.

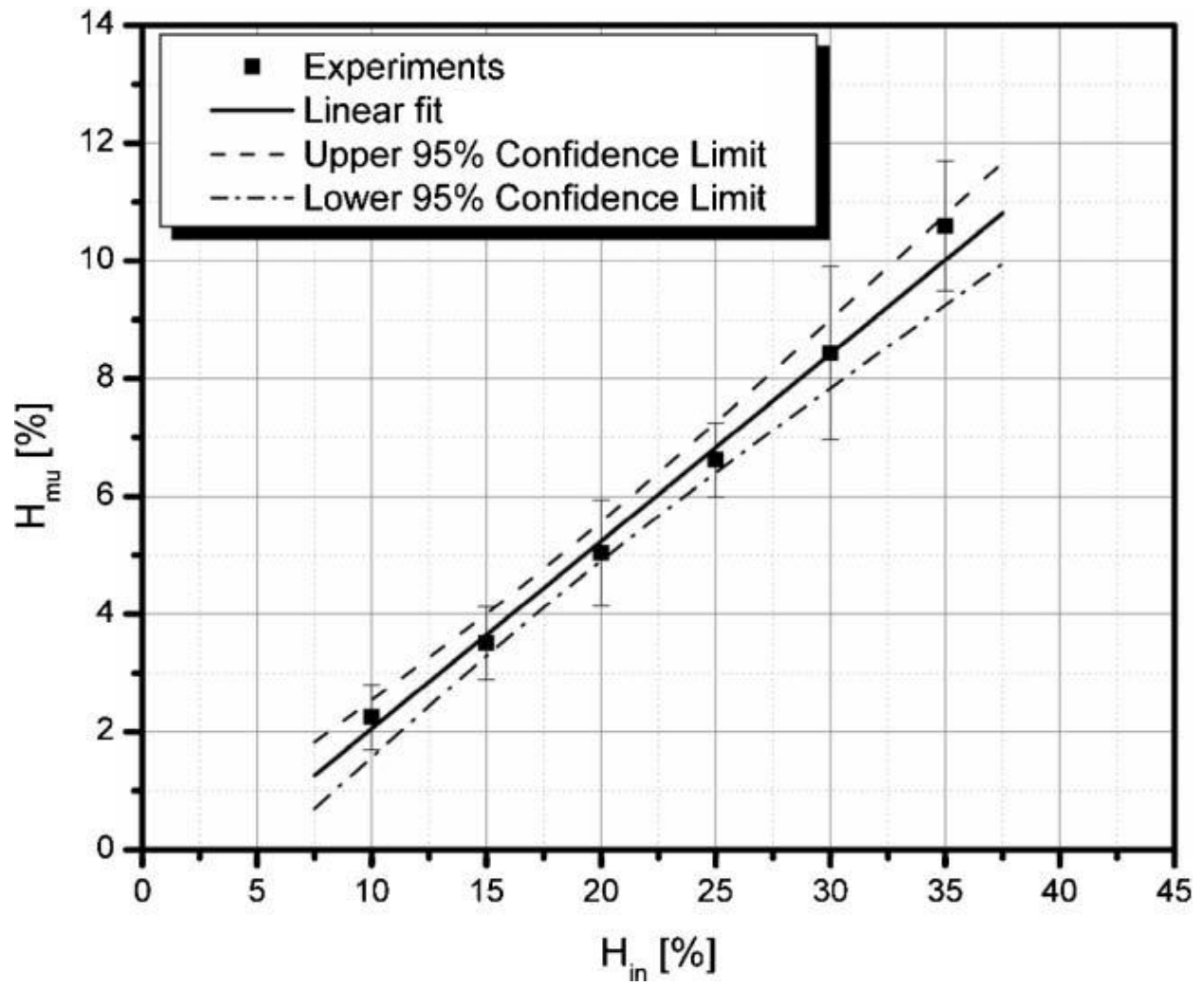


Figure 6.3. Fahreus effect in a 15um microchannel. As inlet hematocrit increases, upstream hematocrit increases.

Chapter 7: White Blood Cell Separation via Immunomagnetic Precipitation

7.1 Introduction

As described in Chapter 5, leukocytes are the only DNA containing cells in human whole blood. Therefore, it is often of interest to a clinician or researcher to analyze the DNA profile of these white blood cells for diagnostic purposes. Doing so requires a blood separation technique capable of producing a nearly pure white blood cell fraction. As described below, an antibody-based method will be implemented on the Diamond HemeSep Cartridge. Antibodies are proteins

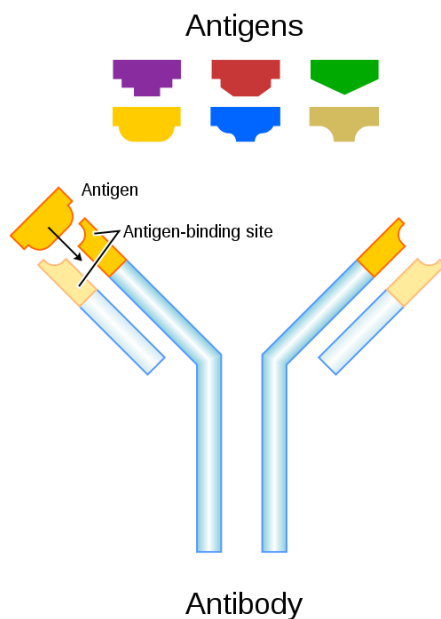


Figure 7.1: Antibodies are proteins that bind their substrates, antigens, much like a key fits in a lock (36)

produced by the white blood cells of the body's immune system. As shown in Figure 7.1, these antibodies contain sites that can bind their substrates, the antigens, much like a lock fits a key (18, 19). The immunomagnetic separation system, therefore, is based on the extreme fidelity of antibody binding to their substrate molecules. Selecting an antibody, chemically linked to a paramagnetic bead, that may bind exclusively to the white blood cells allows for the aggregation and subsequent collection of those white blood cells.

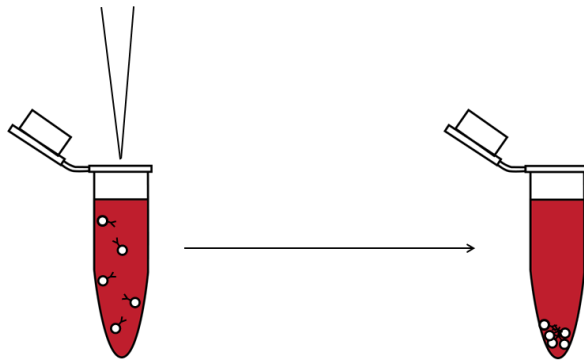


Figure 7.2: Antibodies chemically conjugated to paramagnetic beads facilitate isolation of substrate (white blood cells).

Immunomagnetic separation is quickly becoming a favored technique for efficiently isolating cells from bodily fluids (20). The technique relies on the high affinity of antibodies, proteins produced by the immune system, for their target substrates. The substrates are typically proteins bound to the

membrane of a target cell. As shown in Figure 7.2, when these antibodies are chemically conjugated to paramagnetic beads, they can be magnetically concentrated to the bottom of the well containing the whole blood along with their bound substrate, the white blood cells, facilitating separation of the bead-antibody-substrate from the remainder of the fluid.

7.2 Leukocyte Common Antigen Antibody

7.2.1 Justification for Separation

The five types of white blood cells (neutrophils, basophils, eosinophils, monocytes and lymphocytes as discussed in Chapter 4) are responsible for all immunological functions and are the only nucleated cells in blood. This means that they are the only blood cells containing DNA. As such, separation of these leukocytes is critical for hematological analyses and clinical diagnostic tests for studying progression of disease.

Traditional methods for separating these cells take advantage of differences in buoyant gravity of the different blood components in a density gradient solution such as Ficoll-Paque™ or differences in average diameter between RBCs and WBCs using membrane technology. However, the former method requires careful attention of a human technician and therefore

requires relatively large volumes of blood while the latter is prone to clogging. Therefore, the use of immunomagnetic separation presents an interesting and perhaps more efficient, though slightly more costly method of WBC separation from the rest of the blood components.

7.2.2 Background for Separation Antibody Design

Immunomagnetic separation has several attractive features, including the high degree of specificity of antibody-substrate binding, the kinetics of antibody binding, and the ability to standardize a separation procedure using a liquid handling system.

Antibody-substrate specificity refers to the fidelity of binding between the antibody and its substrate. Ideally for separation purposes, an antibody will bind only to its substrate and not to other molecules, allowing for the most pure substrate to be obtained after magnetic concentration.

Antibodies generally bind quickly and specifically to their target substrates, making them ideal for use in a cartridge such as ours. As shown in Figure 7.3, within about 5 minutes, more

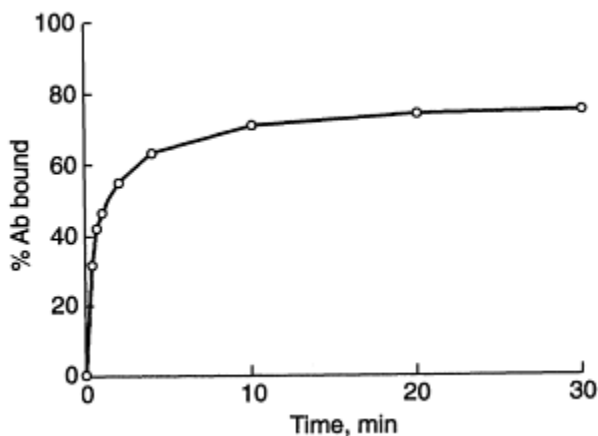


Figure 7.3: Antibodies bind quickly and efficiently to their targets after gentle mixing in a well with human whole blood (21)

than 60% of antibodies can be expected to be bound to their targets after gentle mixing (21). This provides for a quick, reliable and reproducible method for separating out white blood cells from whole blood without having to remove any of the other blood components first. In our process, the total processing time for the white blood cells is

around 13 minutes with only 5 minutes required for the antibodies to bind to the cells during an incubation step as discussed in Chapter 4.

7.3 Magnetic Immunoprecipitation Design

In our chip, we utilize an antibody specific for leukocyte common antigen (LCA, also abbreviated as anti-CD45 antibody). LCA is actually a family of trans-membrane glycoproteins expressed on the surface of all five types of white blood cells discussed in Chapter 4. Five isoforms of LCA, named ABC, AB, BC, B and D have been identified and may be recognized by antibodies known as CD45RA, CD45RB, CD45RC and CD45RD. Our cartridge will utilize a one of these antibodies to target all five isoforms of LCA.

Because LCA is known to be expressed on all white blood cell types, it is an ideal choice for use in our separation, since antibodies specific to the various LCA isoforms may precipitate all types of white blood cells. This simplifies the search for antibodies and increases the yield of white blood cells obtained from the separation. Furthermore, established kits already exist for using such antibodies in separating white blood cells from whole blood. The kit used in the Diamond HemeSep cartridge is the DynaBeads FlowComp Human CD45RA isolation kit from Invitrogen, with a few modifications to the separation procedure as described in Chapter 4.

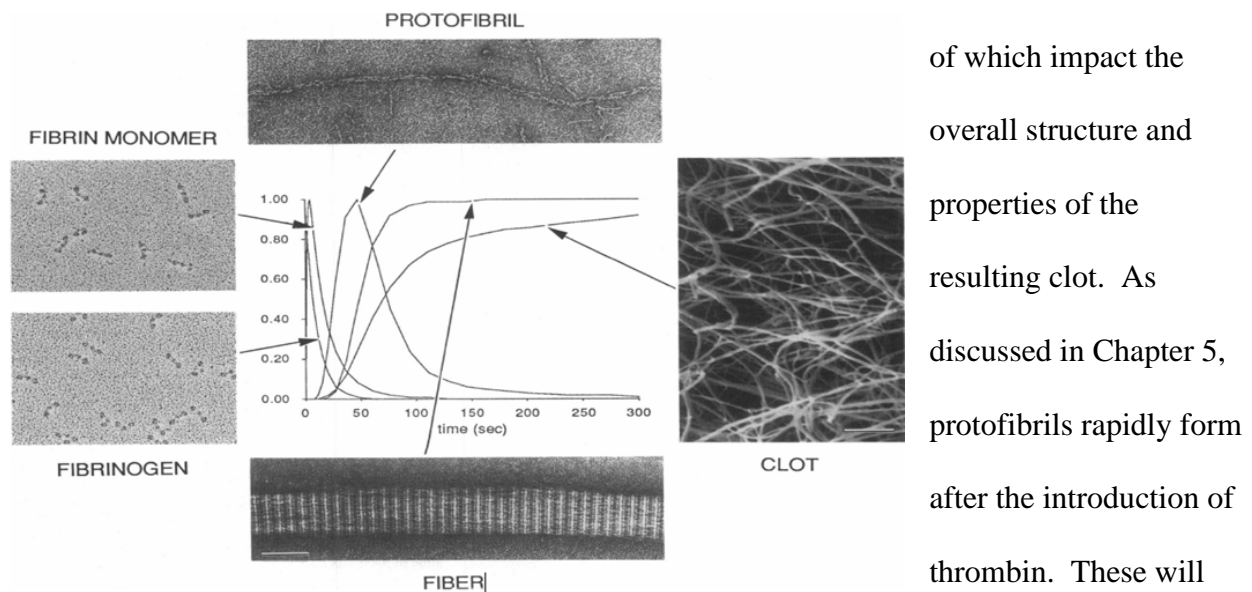
Chapter 8: Serum Separation via Microfiltration

8.1 Introduction

In order to create serum, filtration was the only logical solution. While centrifugation is by far the most utilized technique, a micro-centrifuge in this setup would be impractical. Antibody techniques to pull down fibrin also would not work. Antibodies selective for fibrinogen only remove fibrinogen while leaving all the other clotting factors in the plasma. This produces defibrinated plasma instead of serum. In addition, filters already exist to help keep serum separated from a clot when the sample is spun down in a centrifuge. Thus, a filter system seems quite plausible.

8.2 Physical Characteristics of Fibrin Clot

The design of the filtration system depends on the characteristics of the fibrin clot. Because of the complexity of the reaction to form the clot, different species are present in the plasma at various times. Luckily, the kinetics of this complex series of reactions has been studied extensively (7). Multiple species are created in multiple reactions (Figure 8.1), the rates



of which impact the overall structure and properties of the resulting clot. As discussed in Chapter 5, protofibrils rapidly form after the introduction of thrombin. These will

Figure 8.1: Normalized concentrations of various species against time

grow to an approximate length of 0.8 μ m and a weight of 3,500kDa (22). These are consumed in the production of the much larger fibers that are characteristic of a clot. These fibers grow to a diameter of around 0.2 μ m and lengths on the scale of 100 μ m. Eventually, enough of these fibers are formed to create a gel. If the filtration is timed correctly, it can take place when virtually all of the protofibril has been consumed in production of fiber, but before the onset of the gel.

8.3 Membrane Selection

With these characteristics in mind, a membrane had to be selected. The efficiency of the filtration depends predominantly on the characteristics of the membrane. The first challenge in selecting a membrane is determining the required pore size. The pores of the membrane must be smaller than the particle in order to effectively reject it. In addition, pores for linear proteins/particles must be smaller than those for globular proteins (23). Most of the fibrin will be in the form of very large fibers or aggregated into a gel. This suggests using a large pore size. However, some protofibril may remain. As a result, a pore size of 1 μ m was selected. The use of membranes with this pore size for pre-filtration of serum before its use in easily clogged instruments validates this choice (24).

With pore size selected, the membrane material was chosen. This polymer must be hydrophilic, low-protein-binding and biologically inert. A hydrophilic membrane is used to achieve desired wetting of the membrane by the aqueous solution. In addition, hydrophilic membranes are less likely to adsorb serum proteins. Although several polymers fit these criteria, we selected a 200 μ m thick polysulfone membrane with a 1 μ m pore size. As the membrane by itself is only rated to 30psi, a porous polypropylene backing was selected for the membrane to increase rigidity.

8.4 Flow Rate Calculations

The main difficulty in using filtration is the potential for the membrane to clog. However, the cartridge is single use, so the filter must work only once. The membrane itself offers little resistance, but because fibrin is designed to prevent blood loss, it does an excellent job of impeding flow. The gel that forms on the membrane is the major source of flow resistance. Neglecting the resistance of the membrane, the flow through the fibrin gel can be modeled using Darcy's law.

$$Q = A \frac{k \Delta P}{\mu L} \quad (8.1)$$

Values of 2.5 g/L fibrinogen/L plasma, .001 Pa·s for the viscosity of fibrin and 0.2 g/mL for the density were used (25). The height of the fibrin gel was calculated using the concentration of g fibrinogen/L in plasma and the fibrin density. This height was used as the depth of the bed (L) in Darcy's equation. With a pressure of 50 psi, a flow rate of 59 $\mu\text{L/s}$ was achieved.

Chapter 9: Microfluidic Design Principles

9.1 Introduction

The design of microfluidic devices poses special challenges over their macro-scale counterparts. Because the channel dimensions are no more than a few micrometers in size, highly precise fabrication techniques are needed. Also, since the behavior of fluids is difficult to predict at these length scales, it is important to choose the channel material that offers the least amount of interaction between the working fluid. A widely used microfabrication technique involves the use of photolithography and soft lithography in conjunction with polydimethylsiloxane (PDMS) and is an ideal fabrication technique when working with biological fluids.

9.2 Photolithography

The process of photolithography involves the selective corrosion of a substrate into a desired design. To achieve this, a photomask is designed in a computer-aided drawing (CAD) software package such as Solidworks, which is then laid on top of a silicon wafer coated with a light sensitive substrate (known as the “photoresist”) (26). There are two types of photoresists: positive and negative. A positive photoresist, when exposed to UV light, will be corroded on portions that are covered by the photomask. Alternatively, a negative photoresist will be corroded in areas that are not covered by the photomask (27). Both processes can be seen in Figure 9.1 below.

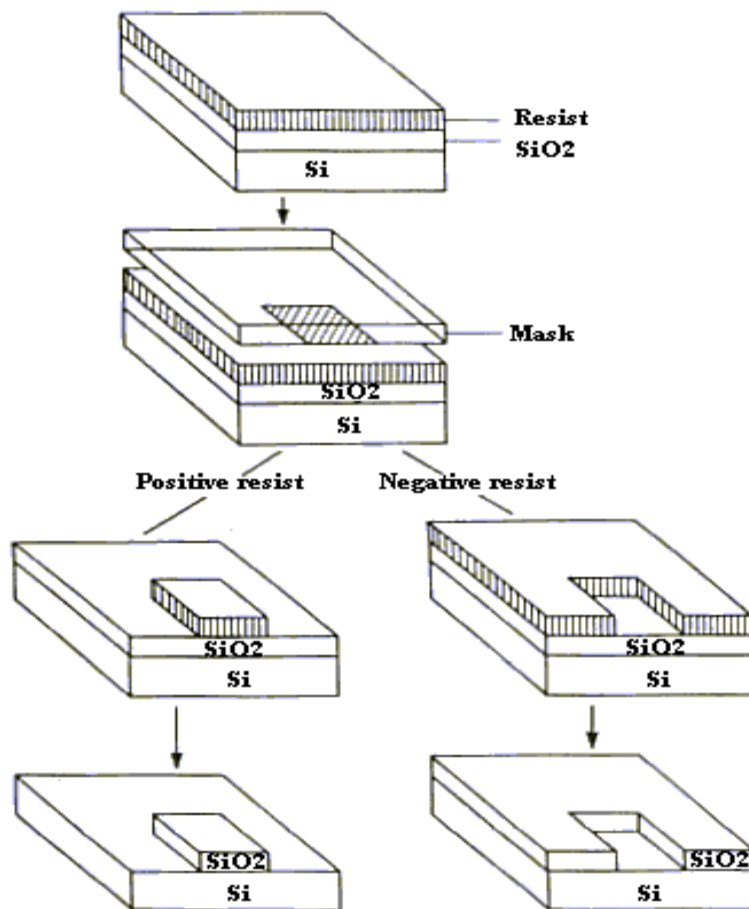


Figure 9.1. Process of photolithography. When exposed to UV light, positive resists are corroded in areas covered by the photomask and negative resists are corroded in areas not exposed by the photomask (27).

Once the photoresist has been completely etched, the entire setup is “hard baked” to harden the photoresist and adhere it to the wafer surface.

9.3 Soft lithography

To prepare the final microfluidic device, soft lithography must be used in conjunction with the “master” mold produced from the photolithography process. Soft lithography involves the use of an elastomeric compound such as PDMS to produce the final design from the master mold via a series of curing steps. First, PDMS is poured over the master mold, where it

solidifies when exposed to high temperatures. The solid PDMS is then peeled off the master mold, resulting in the final microfluidic device (28). This device is then fixed to a glass slide, creating the channels needed for the microfluidic device to work. Figure 9.2 below shows the steps in the soft lithography process.

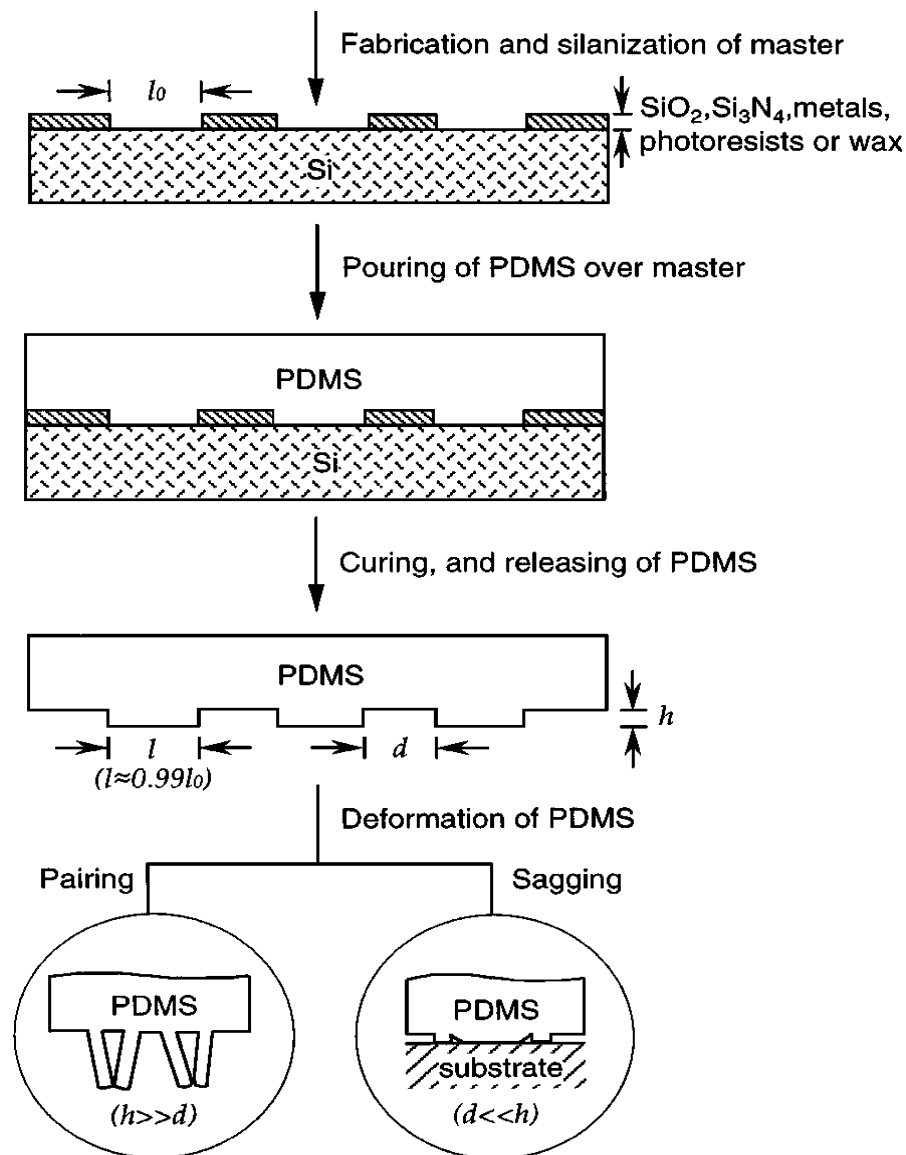


Figure 9.2. Process of soft lithography. Liquid PDMS is poured over the master mold, where it is exposed to high temperatures and cured into a solid. The PDMS is then removed and is used as the final microfluidic device. Some common problems with the soft lithography process include pairing and sagging (28).

9.4 Polydimethylsiloxane

Polydimethylsiloxane (PDMS) is a silicon-based organic compound that is commonly used in microfluidic device designs. It is optically clear, inert, non-toxic, and non-flammable. It has the following chemical design:

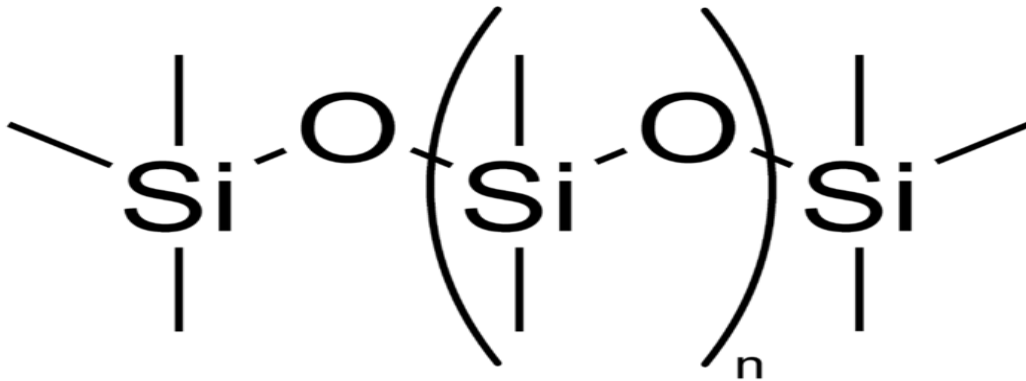


Figure 9.3: Structure of polydimethyl siloxane (PDMS)

In Figure 9.3, n denotes the number of monomers in the compound. The viscosity of PDMS can be increased by increasing the number of monomers (increasing n). This can allow PDMS to take a liquid form (low n) or a rubbery semi-solid (high n) (29). This property makes it a particularly useful elastomer in soft lithography. Other desirable properties of PDMS is that it does not swell with humidity (non-hydroscopic), it has high thermal stability, and it can be easily deformed and reshaped both mechanically and chemically (28).

Chapter 10: Manufacturing Considerations

10.1 Introduction

The Diamond HemeSep Cartridge will primarily be assembled from original equipment manufacturer (OEM) parts. Such parts include the actual plastic cartridge containing the input and output wells, the reagents, and the actual liquid handling unit. The microfluidic portion of the device will be manufactured in our own facilities, originally using manual labor in the prototyping phases and eventually moving towards an automated process with human operators. Originally, we proposed outsourcing microfluidic chip fabrication to an outside company, especially considering the sizeable cost associated with building a manufacturing facility. Ultimately, it seemed most sensible to protect the intellectual property of the cartridge by maintaining complete control over the chip's fabrication.

As mentioned in the previous chapter, the microfluidic chip is composed of two layers, a PDMS layer and a glass layer (a glass slide). The interface between these two layers is what creates the channels through which blood may flow. To create the PDMS layer, soft photolithography replica molding, as described earlier, was decided upon for use.

While all of the other parts are to be obtained from OEMs, assembly still requires accurate and efficient delivery of reagents to the snap-in reagents section of the cartridge. In order to accomplish our eventual goal of 100-200 cartridges per hour, we decided to use a robotic liquid handling system for delivering the reagents to their plastic wells. A major consideration in assembly is storing the reagents at the required conditions. Calcium, stored as CaCl_2 in TBS, may be stored indefinitely in solution at room temperature whereas thrombin must be stored dry at colder temperatures. This means that the user must rehydrate the thrombin prior

to using the cartridge. This also necessitates delivery of the packaged cartridge on dry ice for preservation.

10.2 Fabrication of Microfluidic Device

The microfluidic chip will be composed of two layers including a glass slide and a PDMS layer. PDMS was chosen because of its desirable properties in molding, its optical transparency to wavelengths as low as 300 nm, its good thermal and chemical stability and its low interfacial energy.

Soft photolithography was chosen as a method for fabrication of this PDMS portion of the device. This process was chosen because it can yield a large number of devices with a high fidelity to the “master.” Using a photosensitive resist, as described in Chapter 9, the PDMS layer will be patterned by exposure to UV light. As described, this process begins with the creation of a master, which is fabricated by first creating a resist. Spinning onto a silicon wafer creates this resist and the pattern is created via etching the imprint using UV irradiation. Then, PDMS molds may be created from this master, which may be used on large numbers of chips. Despite its positive features, PDMS has a few known negative attributes, including slight shrinking upon curing and difficulty in accurate patterning below about 10 μm scales. Despite these few negatives, the utility and applicability of soft photolithography made it a desirable process for fabrication of our microfluidic chips.

To fit our goal of 100 – 200 microfluidic chips produced per hour, hundreds of molds will need to be produced for creation of the PDMS portions of the device. For this, only a few masters will need to be created. These masters will be created from normal lithography methods. The channels all have a height of 35 μm , lengths on the order of centimeters and widths ranging

from 15 to 60 μm . These features are all within the size limits allowed by photolithography and can therefore be accurately fabricated using this technique.

In the fabrication process, as described earlier, master creation begins with the application of a positive photoresist onto a silicon wafer and spinning to get a resist thickness of 35 μm . Then, UV light is used to cross link areas not masked that correspond to the channels and reservoirs. After this, the master is baked to cure unexposed resist and the exposed resist is washed with an organic developer that dissolves and etches these areas. This causes the substrate to have channels 35 μm in depth and reservoirs in the positions indicated for our chip. This process is already diagrammed in Figure 9.1 for negative photoresists.

After finishing the master, a negative PDMS mold to the final design pattern may be created (since a positive photoresist was used). Hundreds of PDMS molds, containing many parallel master designs, will be created and used as a stamp. Prior studies have shown that PDMS can be accurately used as a stamp after more than 10 uses (30). This allows each stamp to have a lifetime of up to two months. These parallel molds will be filled with PDMS and then

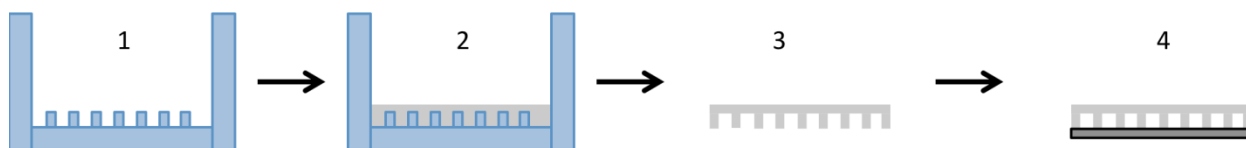


Figure 10.1: This figure displays the manufacturing process involved in creating PDMS chips. (1) A PDMS mold is created from a positive photoresist. (2) This mold is filled with PDMS and cured in an oven at 80 C for 2 hours. (3) the PDMS device is removed from the mold, which may be reused, and is then (4) adhered to a glass slide, creating the channels and reservoirs as indicated in the microfluidic chip design.

thermally cured in a baking process that takes approximately two hours, giving desired PDMS devices with channels and reservoirs as needed to be sealed to the glass slide layer.

We have decided to outsource the creation of the masters while producing the molds and actual devices. Each microfluidic device will require approximately 17.5 mL of PDMS. This assumes less than 1% shrinkage. To accomplish the production of 100-200 chips per hour, we

will begin each day by mixing PDMS with viscous curing agent in a 100 L tank containing 70 L of PDMS. It will need to be mixed for 10 minutes. Then, the mixture will be deposited onto the PDMS molds (blue in Figure 10.1) to create the microfluidic chip (gray in Figure 10.1). The parallel molds will be placed in a curing oven for approximately two hours at 80 °C. Based on the surface area of 4,000 PDMS molds, the oven needs to have a surface area of approximately 60 ft². Based on a prior design project, by Abbot, Lee, Kohli and O'Brien, a suitable curing oven from Wisconsin Oven Corporation, priced at \$22,400 may be used to accommodate the needs of the process.

While the devices cure, reagents are delivered to the plastic cartridge insert as described in the subsequent section. Deposition of the antibody, Dyna-Beads, PBS, lysis buffer, thrombin and calcium will each require about 3 seconds for alignment and subsequent deposition of the liquid, amounting to a total of eighteen seconds. The deposition of the reagents will take place in a cold environment to protect the reagents from degradation and evaporation.

Once the curing has completed, the PDMS chips are taken from the molds via a robotic arm and sealed to glass slides pre-coated with Sigmacote, a hydrophobic material, using a PDMS sealing machine which is estimated to cost \$210,000. From this unit, the chips will be shuttled to an assembly line where a robotic suction arm will assemble the reagent insert and microfluidic chip into the cartridge, then seal the cartridge and transport them into a freezer for delivery.

Our goal will be to produce approximately 100 – 200 cartridges per hour, corresponding to approximately 1 million cartridges per year.

10.3 Robotic Liquid Handling System

Although most cartridge parts will be obtained from OEMs, reagents will still need to be delivered to the cartridge reagent insert prior to packaging. To accomplish the delivery of

microliter volumes of reagent, a few different options were considered. Namely, we considered the use of an inkjet handling system and a robotic pin tool. While both of these technologies are capable of accurately and reproducibly delivering small quantities of liquid on the nanoliter scale, their use in microliter quantities is far too slow and costly to be of great use. Instead, we opted to use a robotic liquid handling system. This system is capable of delivering each reagent to the appropriate well in approximately 1-2 seconds, allowing us to realize our goal of manufacturing 100-200 cartridges per hour. Since our design is based off of a standard 96 well-plate micro-assay design, standard liquid handling robots sold may be used to insure accurate deposition of reagents. This will work by passing the cartridge insert along a conveyer belt underneath the robotic liquid handling unit, where the unit may be programmed to deliver the reagents to the standard well plate locations. Such a unit is estimated to cost \$35,000 and is assumed to be able to deliver the reagents in 3 seconds per transfer.

Because of the relatively large volumes (μl scale) of reagents being deposited into the wells, evaporation of reagents is of little concern. According to the literature, approximately 1 nl of aqueous solution may be assumed to evaporate in approximately 30 seconds. However, precautions to reduce the risk are to include more reagent than is necessary during cartridge operation and to seal the reagent wells with foil shortly following deposition. This problem will be addressed as needed during the first year of testing.

10.4 Manufacturing Schedule

Using an assumed market size of 1 million chips per year, which is roughly equivalent to the number of blood draws taken in clinical trials every year, and a market capture of approximately 50%, we will need to produce 500,000 chips per year.

To meet this market capture goal, there are several considerations and assumptions to make. First, we have assumed that the manufacturing facility can operate 8 hours a day, 5 days a week for 50 weeks of the year (250 total days). Therefore, the number of chips needed for sale is given by equation 10.1:

$$\frac{500,000 \text{ chips per year}}{250 \text{ days of operation per year}} = 2,000 \text{ chips produced per day} \quad (10.1)$$

Furthermore, it is assumed that roughly 25% of chips manufactured will be used for quality control testing and therefore, an additional number of chips must be produced. Of these chips, it is assumed 25% of them will be defective, such that the requisite number of chips is given by equation 10.2:

$$\text{chips} = \frac{2,000 \text{ chips per day}}{(0.75)^2} = 3556 \approx 4000 \text{ chips per day} \quad (10.2)$$

This schedule assumes manufacturing starts at time $t = 0$ (9:00 AM). Mixing and deposition of PDMS onto the molds is represented by the box labeled “mix/dep”. Mixing takes approximately 10 minutes and each deposition is assumed to take 3 seconds. It is assumed that 10 chips’ worth of PDMS can be deposited to the molds per deposition yielding the following amount of time necessary for the step:

$$\frac{4000 \text{ chips} \cdot 3 \text{ seconds per deposition}}{10 \text{ chips per deposition}} = 20 \text{ minutes} \quad (10.3)$$

The chips are then cured in an oven for a total time of 2 hours. Simultaneously, the reagents are deposited into the cartridge using the times discussed above:

$$\frac{2 \text{ seconds}}{\text{reagent}} \cdot \frac{6 \text{ reagents}}{\text{chip}} \cdot \frac{4000 \text{ chips}}{\text{day}} = 13.4 \text{ hours} \quad (10.4)$$

Because this time exceeds the typical work day, two liquid handling robots will be run in parallel for a total of 7 hours each. Note that these calculations are for the final year of

production, and that manufacturing facilities can be modified as necessary during actual production.

After removing the PDMS layers from the oven, they need to be sealed to the glass layer to complete the microfluidic device. This process is estimated to take 8 hours (4 hours if split into two batches) and may begin as soon as the PDMS layers are removed from the oven (i.e., simultaneously with reagent loading). Doing so saves time during the process.

Subsequently, the cartridge needs to be assembled and packed. This process is estimated to take roughly 4 hours (for two batches) and may begin midway through the reagent deposition as inserts become available to be assembled into cartridges. Doing so allows the cartridges to be assembled over the course of a typical workday as displayed in Figure 10.2 below.

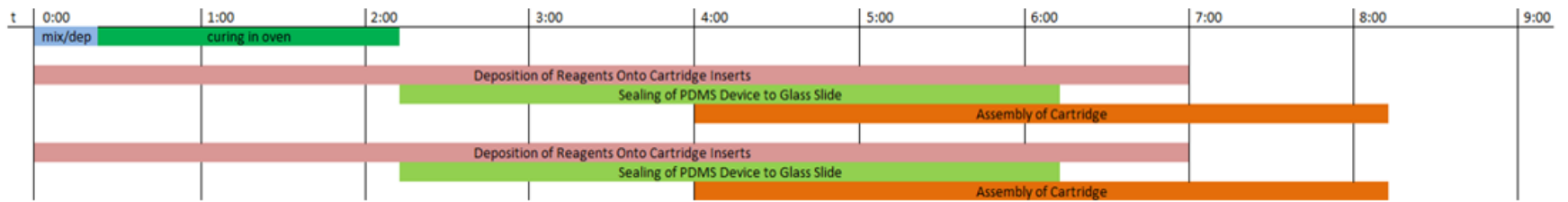


Figure 10.2: Scheduling for the manufacturing process

Chapter 11: Diamond HemeSep Development Timeline

11.1 Introduction

After completing the design stage, we project that the Diamond HemeSep cartridge can be marketed for use within a year. This time is primarily required for acquiring FDA approval for the device, although part of the time will be simultaneously used for research and development to improve on design weaknesses discussed in the report.

11.2 Product Development and Prototyping

We have allocated one year for seeking FDA approval and further optimization of the cartridge system. Preliminary experimental results showed that the device can successfully skim plasma from whole blood, but further tuning is required. During this time period, scientists and engineers will need to address a few key issues to insure the success of the project. Specifically, experimental results suggested that the channel dimensions chosen can handle flow rates as high as 25 $\mu\text{L}/\text{min}$ as opposed to the 10.5 $\mu\text{L}/\text{min}$ assumed previously.

11.3 FDA Approval

The Diamond HemeSep Cartridge is considered to be a medical device, necessitating that FDA approval be obtained before the product may begin to be distributed to hospitals and clinical research facilities. As is the case with obtaining FDA approval for any such device, we must first classify what class the device falls into. Class I devices include basic medical instruments whose proper functioning can be determined from inspection and whose failure poses no harm to the patient. Class III devices are devices whose failure could pose significant or life threatening harm to the patient. An example of a Class I device includes band-aids

whereas a Class III device would be something like a pacemaker. The Diamond HemeSep cartridge seems to fall in the middle of these two extremes under Class II (31). The device has a potential to fail so its safety and efficacy must be substantiated. However, failure of the device poses no direct threat to the patient. To verify this product classification, product design specifications, manufacturing protocols and prototypes must be sent to the FDA. After obtaining this verification, the appropriate paperwork can be filed and clinical trials can commence.

11.3.1 Clinical Trials

In acquiring FDA approval for the device, it will need to be proved that the Diamond HemeSep Cartridge is both safe and effective. To this end, it may be most beneficial to try and prove Substantial Equivalence. According to the FDA, proving substantial equivalence means that, in comparison to a predicate product, the new product: has the same intended use and different technological characteristics than the predicate, but that do not raise new questions of safety and effectiveness.

The safety and effectiveness of the Diamond HemeSep Cartridge will be verified through a series of clinical trials. Test subjects in each Phase of the trials will have multiple blood samples collected, some of which will be analyzed using conventional centrifugation and the remainder of which will be processed using the Diamond HemeSep. The former samples will serve as controls against the latter.

11.3.2 Phase I

Phase I is estimated to take 1 month to run and analyze. Its main purpose will be prove that the Diamond HemeSep cartridge is safe and does not show a variance in blood plasma purity, serum purity or DNA profile obtained from blood processing when compared to

conventional laboratory methods such as centrifugation. To this end, 75 test subjects will be used. Each subject will have two 5 mL blood samples drawn and tested using both centrifugation and the Diamond HemeSep. Then, the obtained blood fractions will be examined using a smear technique for determining plasma and serum purity and the DNA profiles obtained from the WBC samples will be compared using the centrifuged sample as a control.

11.3.3 Phase II

Phase II will take approximately 5 months to complete and will primarily serve to prove the efficacy of the device as well as continued safety. To do so, the number of test subjects will be increased to approximately 300 with each subject being tested three times at one week intervals as described before. The blood will be tested as in phase I and the results will be compiled and analyzed for variation in the results due to the different separation methods.

11.3.4 Phase III

Phase III has an estimated duration of 6 months. Its purpose is to prove that the device is ready for distribution and use in clinical research facilities. In this trial, the number of subjects will increase to approximately 3000 with each subject being tested exactly as before but for a total of four times at one week intervals. As before, the data will be compiled and analyzed.

11.4 Preparation for Manufacturing

After successful results have been obtained from the clinical trial series and while waiting for FDA approval, manufacturing facilities will be set up. Production space will be leased and the parts obtained from OEMs will be assembled into Diamond HemeSep Cartridges once FDA clearance has been received.

Chapter 12: Financial Analysis

12.1 Market Projection

We have projected the company's expected revenues over a reasonable time horizon of 10 years and also assumed a product life of 10 years for our base case analysis. Research and development will commence in 2013 and production will begin in 2014. From consulting with industry professionals at Becton Dickinson, a reasonable base case price for the cartridge and the processing unit are \$25 and \$100,000, respectively. An inflation rate of 2.5% was assumed. This was based on average annual inflation rate of 2.5% from 2002 to 2012 (32). We have assumed that the Diamond HemeSep will reach a market share of 50% by the third year of production and that this share will be maintained for subsequent production years.

Table 12.1 shows total annual revenues. Complete market projections calculations can be found in Appendix 16.5.1.

End of Year	Total Revenues
2013	\$0
2014	\$20,275,781
2015	\$46,284,540
2016	\$84,295,269
2017	\$30,141,247
2018	\$22,872,920
2019	\$24,734,204
2020	\$26,746,950
2021	\$28,923,483
2022	\$31,277,131
2023	\$33,822,308

Table 12.1 Total revenues with base case assumptions

12.2 Costs Sheet

The costs of reagents have been confirmed with sales representatives from suppliers and a 25% discount was applied to take into account of bulk purchasing prices. Per Dr. Diamond and consultants, the costs of the microfluidic chip, micro-filter, and magnet are estimated as \$5 per chip, \$1 per well, and \$100 per well, respectively.

Costs of manufacturing the liquid handler from OEM's have been estimated as \$11,500. This figure was estimated from considering a price quote of \$75,000 from sales representatives of Becton-Coulter and assuming an 85% mark-up. This estimate lies within the ball-park of estimates from Dr. Diamond and industry consultants.

Costs of manufacturing machinery and equipment are estimated in Chapter 10.

Laboratory and corporate space costs were estimated at \$10/ft²/month and manufacturing space costs were estimated at \$8/ft²/month. Salaries were based on estimates by the design team (2, 4-6, 32, 33).

Clinical trials are estimated at \$2.5 MM for material and human costs. FDA approval, including application and legal fees is estimated to cost approximately \$500,000 (2, 4-6, 32, 33).

R&D start-up costs are a lump-sum to cover materials, utilities, extra equipment, and anything else our scientists might need. These costs are assumed to be approximate \$1 MM per year pre-production. Once production has begun, 5% of revenues will be re-invested in R&D so that product offerings can be improved and expanded. Lab information management software cost was assumed to be \$100,000 (2, 4-6, 32, 33).

Cost estimations are detailed in Appendix 16.5.2.

12.3 Operating Assumptions

Sales, General, and Administrative costs (SG&A), also known as overhead, are assumed to be a percentage of revenues. Due to a learning curve, overhead functions are expected to become more efficient over time, so SG&A costs are expected to reduce in subsequent years.

Estimates of laboratory space were based on 250 sq. ft/person. Estimates for corporate space were based on 100 sq. ft. per person in management and 75 sq. ft. per person for all other employees. The number of employees required is based on the design team's best estimates for equipment operation and company growth (2, 4-6, 32, 33).

The number of pieces for each process machine is based on manufacturing considerations and is justified in Chapter 10. Operating assumptions are detailed in Appendix 16.5.3.

12.4 Inventory, Working Capital, and PP&E

Inventory was found by summing the total cost of the cartridges and the processing units needed for that particular year. Since all costs were given in 2012 dollars, adjustments for inflation were made.

Working capital is found by subtracting current liabilities from current assets. Current assets are calculated by the sum of accounts receivable and inventory. Accounts receivable is based on the outstanding amount owed to the company by customers that have not yet paid for products that is calculated by multiplying total revenues by 1/12, or 8.33%, which is thirty days out of the year. Inventory is typically held for seven days. To find the working capital cost of holding on to this inventory, total revenues in a particular year are multiplied by 1.92%, which is seven days out of the year. Current liabilities consist of accounts payable and cash reserves for salaries. Accounts payable is the outstanding amount that a company owes to a supplier for

goods that have been delivered. Accounts payable is calculated by multiplying the cost of inventory, depreciation, maintenance capital expense (capex), and overhead by 8.33%, which accounts for thirty days to pay suppliers. Cash reserves for salaries are the amount of cash needed to pay all employees for one month. Additional working capital for year n is found by subtracting the working capital for year n-1 from the working capital needed for year n. This represents a change in free cash flow and can be found as a line item in the free cash flow section.

Net Plant, Property, and Equipment expenses were calculated by summing the beginning account, purchases, and maintenance capex and subtracting the accumulated appreciation. Since the Diamond HemeSep is leasing laboratory, production, and corporate space, these costs are not included in PP&E. Equipment purchases are the cost of all equipment purchased in a given year. Maintenance capex consists of equipment needed to maintain previously purchased equipment and machinery. Maintenance capex was estimated to be 10% of the total cost of equipment from previous years.

Depreciation was found using the MARCS depreciation schedule. The Diamond HemeSep's equipment falls into a class life of 10 years. The basis for depreciation was found by summing the equipment purchases and the maintenance capex for the respective year.

The breakdown of inventory, working capital, and PP&E are detailed in Appendix 16.5.4.

12.5 Income Statement

The income statement shows how the Diamond HemeSep's revenues are transformed into net income. Cost of goods sold (COGS), which are the direct costs attributable to the production of goods, are subtracted from Revenues to arrive at gross profit. Next, indirect

expenses are subtracted from gross profit to arrive at earnings-before-interests-and-taxes (EBIT).

To find net income, a typical 37% tax rate was assumed. If the EBIT is negative, a tax benefit can be applied often referred to as a deferred tax asset. The negative earnings are not taxed, but instead they are carried forward and deducted from positive taxable income in the future.

Assuming Diamond HemeSep has no debt, taxes are subtracted from EBIT to arrive at Net Income. Appendix 16.5.5 provides a thorough breakdown of the income statement.

End of Year	Gross Profit	% Margin	EBIT	% Margin	Net Income	% Margin
2013	\$ -	0%	\$ (5,048,125)	0%	(\$5,048,125)	0%
2014	\$ 15,838,316	78%	\$ 10,826,102	53%	\$ 8,688,250	43%
2015	\$ 36,687,412	79%	\$ 29,173,940	63%	\$ 18,379,582	40%
2016	\$ 66,998,446	79%	\$ 55,824,601	66%	\$ 35,169,499	42%
2017	\$ 18,976,395	63%	\$ 12,281,591	41%	\$ 7,737,402	26%
2018	\$ 10,799,528	47%	\$ 4,795,796	21%	\$ 3,021,351	13%
2019	\$ 11,678,340	47%	\$ 5,377,751	22%	\$ 3,387,983	14%
2020	\$ 12,628,665	47%	\$ 6,040,052	23%	\$ 3,805,233	14%
2021	\$ 13,656,322	47%	\$ 6,810,615	24%	\$ 4,290,687	15%
2022	\$ 14,767,605	47%	\$ 7,656,240	24%	\$ 4,823,431	15%
2023	\$ 15,969,319	47%	\$ 8,573,781	25%	\$ 5,401,482	16%

Table 12.2: Net income statement for the Diamond HemeSep blood processing unit.

12.6 Free Cash Flow

Free cash flow represents the cash that a company is able to generate after the expenses required to maintain or expand its asset base has been paid. Free cash flow is a basis to determine the valuation of a company using the Discounted Cash Flow method. It is calculated as operating cash flow minus capital expenditures. Operating cash flow is net income plus adjustments for non-cash items such as depreciation and working capital. Capital expenditures are assumed to be purchases of PP&E (2, 4-6, 32-34). The free cash flow generated each year are shown in Appendix 16.5.6.

12.7 Valuation and Returns

The value of the Diamond HemeSep is determined by the Discounted Cash Flow method. Free cash flow was projected over the ten year horizon. A terminal value was determined at the end of year ten by using the perpetuity formula and assuming a conservative growth rate of zero. The free cash flows for each year and the terminal value are then discounted to present value using a discount rate.

The discount rate was determined using the Capital Asset Pricing Model (CAPM) and assuming that the ownership of Diamond HemeSep is 100% equity based. Risk free rate was estimated using 10 year Treasury bill rate. A typical market risk premium of 7% was assumed. Since the Diamond HemeSep is a novel medical device, a relatively risky venture, a typical beta for this industry of 2 was assumed. Using CAPM, the equity discount rate is 16%.

$$CAPM: r_e = r_f + \beta (r_m - r_f)$$

We calculated the Internal Rate of Return (IRR) for investors. A company usually goes through multiple rounds of financing. Since Diamond HemeSep is a product-focused venture with relatively fast go-to-market time, we have considered one round of financing to estimate IRR. Series A financing is the first round of financing undergone for a new business venture and is typically the first time that company ownership is offered to external investors, such as venture capital and angel investors. Financing is assumed to be provided in the form of convertible preferred stock (2, 4-6, 32-35).

The NPV of Diamond HemeSep is approximately \$51MM and its IRR is approximately 45%. The IRR is greater than the discount rate and falls within investor's preference of 30%-

50% IRR for risky companies. Table 12.3 provides a summary of assumptions and results of the valuation analysis. A thorough breakdown of the valuation analysis is shown in Appendix 16.5.7.

Perp. Growth rate	0.00%
Risk Free Rate (10 year treasury bill)	2.23%
Market Risk Premium	7.00%
Beta	2
Discount Rate	16.23%
NPV	\$50,601,988
IRR to Series A Investor	45%

Table 12.3: NPV and IRR for a Series A investor of the Diamond HemeSep blood processing unit.

12.8 Payback period

The payback period is the length of time required to recover the cost of an investment. Usually, the goal of a start-up company is to achieve payback period of two years after production (2, 4-6, 32-35).

Two methods of calculating the payback period can be used – simple or discounted. The simple payback period ignores the time value of money, whereas the discounted payback period takes into account the time value of money. Cumulative present wealth (PW) is calculated from summing the free cash flows. The payback period occurs when the cumulative PW goes from negative to positive. While the discounted method is used more often, some analysts still like to see both methods.

For the Diamond HemeSep, the payback period under the simple and the discounted methods are one and two years respectively after production has begun. Appendix 16.5.8 provides further details on the calculation behind the payback period.

12.9 Sensitivity Analysis

Sensitivity analysis was performed to study how NPV and IRR fluctuated with respect to the following variables: market share, price, and number of clinical trial years. For each variable, worst and best case scenarios were considered.

12.9.1 Sensitivity to Market Share

The base case assumes market share of 15%, 30%, 50% in first three years of production and market share saturation at 50%. While this assumption is ambitious, we believe that the Diamond HemeSep is a novel technology that is capable of disrupting the market, has little competition, and could become an industry standard. However, these values may change depending on other factors such as later entrants by competitors and sensitivity analysis was performed to determine the impact on NPV and IRR of the company. Our analysis shows that the NPV and IRR are quite sensitive to market share. Under the worst case scenario, if the Diamond HemeSep only achieves a market penetration rate of 25%, the NPV will be \$12MM and the IRR will be 20%. The company would still be profitable, but the investment is certainly not as attractive. On the other hand, our analysis also shows that the Diamond HemeSep has the potential to increase value through increased market share. Under the best case scenario, if the Diamond HemeSep continues to grow its market share at 5% growth rate after 50% penetration, the NPV will be approximately \$69MM and the IRR will be 51%. This analysis demonstrates the importance of market share. Thus, Diamond HemeSep should try to leverage its first-mover advantages and aggressively solidify market share early on.

% Change in Market Share from Base Year				
	NPV	% Change	IRR to Series A	% Change
Worst Case				
0,5,10,15,20,25,25,25,25,25	\$11,858,888	-77%	20%	-55%
0,10,20,30,30,30,30,30,30,30	\$24,845,293	-51%	32%	-29%
Base Case				
0,15,30,50,50,50,50,50,50,50	\$50,601,988	0%	45%	0%
Best Case				
0,15,30,50, grows at 3%	\$60,630,408	20%	48%	8%
0,15,30,50,grows at 5%	\$68,526,272	35%	51%	14%

Table 12.4: Sensitivity to Market Share for the Diamond HemeSep blood processing unit

12.9.2 Sensitivity to Price

The base case assumes that the cartridge will sell for \$25 and \$100K for the processing unit. These prices can vary due to competition and other market dynamics. Our sensitivity analysis shows that the NPV and IRR still fall within reasonable and attractive values in worst case scenarios.

	NPV	% Change	IRR to Series A	% Change
Worst Case				
-20% change in price	\$28,052,057	-45%	34%	-24%
-10% change in price	\$39,327,023	-22%	40%	-11%
Base Case				
0% change in price	\$50,601,988	0%	45%	0%
Best Case				
+10% change in price	\$61,876,954	22%	49%	9%
+20% change in price	\$73,151,919	45%	52%	17%

Table 12.5: Sensitivity to Price for the Diamon HemeSep blood processing unit.

12.9.3 Sensitivity to Number of Clinical Trial Years

Under base case assumptions, the Diamond HemeSep will undergo one year of clinical trials. The actual amount of time required for clinical trials and FDA approvals could vary due to delays and setbacks. Since the Diamond HemeSep falls under Class II Medical Device category,

it has a relatively short clinical trial period. In worst case scenario, the Diamond HemeSep might undergo one additional year of clinical trials, which will result in an NPV of \$34MM and IRR of 37%.

	NPV	% Change	IRR to Series A	% Change
Base Case				
1 year	\$50,601,988		45%	
Worst Case				
2 years	\$34,371,999	-32%	37%	-16%

Table 12.6: Sensitivity to an additional clinical trial year for the Diamond HemeSep blood processing unit

12.10 Summary

Overall, the Diamond HemeSep is expected to be a profitable venture. Under base case assumptions, the Diamond HemeSep can generate a positive NPV of \$50MM and investors can expect an approximate IRR of 45%. The Diamond HemeSep's payback period is short and expected to be within one or two years after production. These metrics suggest that the Diamond HemeSep is a highly attractive investment.

Our sensitivity analysis revealed that the Diamond HemeSep's NPV and IRR are quite sensitive to market share. Our base case assumption that the Diamond HemeSep will achieve market penetration of 50% after 3 years of production is ambitious, but justified from our analysis of the market dynamics. Thus, the Diamond HemeSep should take full-advantage of its first-mover position and secure as much market share early on as possible.

Chapter 13: Recommendations and Conclusions

The realization of the Diamond HemeSep cartridge system promises to revolutionize the healthcare industry and provide researchers and physicians with a powerful and efficient diagnostic tool. However, the design proposed in this report will require adaptations and enhancements if it is to reach its full potential.

Although our microfluidic design achieved positive results in the lab, modifications must be made to the device geometry to increase its plasma separation efficiency. Specifically, the flow rate resistance ratios will need to be increased by either elongating the microfluidic channels or providing additional micro-valves to increase the pressure drops across the daughter channel. Another largely unproven component of the Diamond HemeSep cartridge is our serum separation apparatus. Although the laws that govern the filtration process are well known, the physical behavior of the device cannot be predicted with complete accuracy and will require laboratory testing before it can be confidently implemented in the cartridge. These modifications and testing would be expected to occur during the yearlong research and development stage of our manufacturing process.

Further study will also need to be focused on proving the efficacy of the Diamond HemSep cartridge compared to traditional centrifugation processes. Specifically, the composition and purity of the cartridge outputs must be on par with existing technology and the reproducibility of the cartridge outputs must be demonstrated as well.

Although our market and economic analysis focuses solely on the research and development segment, clinical uses of the Diamond HemSep cartridge system can be expected as well. For instance, it has been found that fatal inflammatory responses can occur in

postoperative patients that undergo procedures involving cardiopulmonary bypass. These responses include the secretion of pro-inflammatory cytokines into the blood stream along with other measurable blood changes. By implementing the Diamond HemeSep system in hospitals, patients' blood can be analyzed in real-time during the procedure to quickly respond to and treat this inflammatory response.

Chapter 14: Acknowledgements

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Chapter 16: APPENDIX**16.1 MSDS Reports***16.1.1 PDMS*

Material Safety Data Sheet

Poly(dimethylsiloxane)

ACC# 95130

Section 1 - Chemical Product and Company Identification

MSDS Name: Poly(dimethylsiloxane)**Catalog Numbers:** AC178440000, AC178442500, AC178445000**Synonyms:** Simethicone; Dimethicone.**Company Identification:**

Acros Organics N.V.

One Reagent Lane

Fair Lawn, NJ 07410

For information in North America, call: 800-ACROS-01**For emergencies in the US, call CHEMTREC:** 800-424-9300

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
9016-00-6	Poly(dimethylsiloxane)	100	unlisted

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: clear liquid.

Caution! May cause eye, skin, and respiratory tract irritation. The toxicological properties of this material have not been fully investigated.

Target Organs: None known.

Potential Health Effects

Eye: May cause eye irritation.

Skin: May cause skin irritation. May be harmful if absorbed through the skin.

Ingestion: May cause irritation of the digestive tract. May be harmful if swallowed.

Inhalation: May cause respiratory tract irritation. May be harmful if inhaled.

Chronic: Adverse reproductive effects have been reported in animals. Animal studies have reported the development of tumors.

Section 4 - First Aid Measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develops, get medical aid.

Skin: Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists.

Ingestion: Do not induce vomiting. Get medical aid if irritation or symptoms occur.

Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.

Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear.

Extinguishing Media: Use water spray, dry chemical, carbon dioxide, or appropriate foam.

Flash Point: > 100 deg C (> 212.00 deg F)

Autoignition Temperature: Not applicable.

Explosion Limits, Lower: Not available.

Upper: Not available.

NFPA Rating: (estimated) Health: 1; Flammability: 1; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Provide ventilation. Do not let this chemical enter the environment.

Section 7 - Handling and Storage

Handling: Use with adequate ventilation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation.

Storage: Store in a cool, dry place. Store in a tightly closed container.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.

Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Poly(dimethylsiloxane)	none listed	none listed	none listed

OSHA Vacated PELs: Poly(dimethylsiloxane): No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment

Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin: Wear appropriate protective gloves to prevent skin exposure.

Clothing: Wear appropriate protective clothing to prevent skin exposure.

Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Section 9 - Physical and Chemical Properties

Physical State: Liquid
Appearance: clear
Odor: odorless
pH: Not available.
Vapor Pressure: Not available.
Vapor Density: Not available.
Evaporation Rate:Not available.
Viscosity: 100 cSt @ 25 deg C
Boiling Point: > 65 deg C @ 760 mmHg
Freezing/Melting Point:Not available.
Decomposition Temperature:Not available.
Solubility: Insoluble.
Specific Gravity/Density:0.965
Molecular Formula:Not available.
Molecular Weight:Not available.

Section 10 - Stability and Reactivity

Chemical Stability: Stable under normal temperatures and pressures.
Conditions to Avoid: Incompatible materials, excess heat.
Incompatibilities with Other Materials: Strong oxidizing agents, strong acids, strong bases.
Hazardous Decomposition Products: Carbon monoxide, carbon dioxide, silicon dioxide.
Hazardous Polymerization: Will not occur.

Section 11 - Toxicological Information

RTECS#:
CAS# 9016-00-6: TQ2690000
LD50/LC50:
Not available.

Carcinogenicity:
CAS# 9016-00-6: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: Tumorigenic effects have been reported in experimental animals.
Teratogenicity: No information found
Reproductive Effects: Adverse reproductive effects have occurred in experimental animals.

Mutagenicity: No information found**Neurotoxicity:** No information found**Other Studies:**

Section 12 - Ecological Information

Ecotoxicity: Fish: Rainbow trout: LC50 > 10000 mg/L; 96 Hr; Unspecified Fish: Bluegill/Sunfish: LC50 > 10000 mg/L; 96 Hr; Static bioassay Based on the Koc values, this substance will be immobile in soil and is expected to adsorb to particulates and organic matter in the water column. Rapid and extensive degradation is expected on dry surface soils. Some microbial degradation of small compounds is likely. High molecular weight poly(dimethylsiloxane) may bioconcentrate in aquatic organisms.

Environmental: Poly(dimethylsiloxane) with lower molecular weights exist in the atmosphere in the vapor and particulate phases. Those with higher molecular weights exist solely in the particulate phase. Particulate phase poly(dimethylsiloxane) will be removed from the atmosphere by dry deposition while vapor phase poly(dimethylsiloxane) will be degraded by the reaction with photochemically-produced hydroxyl radicals with a half-life of 32 hours.

Physical: No information available.**Other:** Do not empty into drains.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.

RCRA P-Series: None listed.**RCRA U-Series:** None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	Not regulated.	Not regulated.

Hazard Class:		
UN Number:		
Packing Group:		

Section 15 - Regulatory Information

US FEDERAL

TSCA

CAS# 9016-00-6 is not listed on the TSCA inventory. It is for research and development use only.

Health & Safety Reporting List

None of the chemicals are on the Health & Safety Reporting List.

Chemical Test Rules

None of the chemicals in this product are under a Chemical Test Rule.

Section 12b

None of the chemicals are listed under TSCA Section 12b.

TSCA Significant New Use Rule

None of the chemicals in this material have a SNUR under TSCA.

CERCLA Hazardous Substances and corresponding RQs

None of the chemicals in this material have an RQ.

SARA Section 302 Extremely Hazardous Substances

None of the chemicals in this product have a TPQ.

Section 313 No chemicals are reportable under Section 313.

Clean Air Act:

This material does not contain any hazardous air pollutants.

This material does not contain any Class 1 Ozone depleters.

This material does not contain any Class 2 Ozone depleters.

Clean Water Act:

None of the chemicals in this product are listed as Hazardous Substances under the CWA.

None of the chemicals in this product are listed as Priority Pollutants under the CWA.

None of the chemicals in this product are listed as Toxic Pollutants under the CWA.

OSHA:

None of the chemicals in this product are considered highly hazardous by OSHA.

STATE

CAS# 9016-00-6 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations**European Labeling in Accordance with EC Directives****Hazard Symbols:**

Not available.

Risk Phrases:**Safety Phrases:**

S 24/25 Avoid contact with skin and eyes.

WGK (Water Danger/Protection)

CAS# 9016-00-6: No information available.

Canada - DSL/NDSL

CAS# 9016-00-6 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of D2B.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

Canadian Ingredient Disclosure List**Section 16 - Additional Information**

MSDS Creation Date: 5/14/1999

Revision #4 Date: 1/11/2008

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

16.1.2 Thrombin



Manufactured By: HYPHEN BioMed

MATERIAL SAFETY DATA SHEET

ENGLISH**1. IDENTIFICATION**

Name	Product number	Presentation
Human Thrombin	AEZ006A	100 NIH
	AEZ006B	1000 NIH
	AEZ0060	10 NIH
	AEZ006K	Set of 6x 10 NIH
	AEZ006L	Set of 10 x 100 NIH

Application / Intended use

It must be used according strictly to the instructions of package insert, and for the indicated purpose.

Manufacturer: HYPHEN BioMed
155 rue d'Eragny
95000 Neuville sur Oise (France)
Tel: 33.1.34.40.85.10
Fax: 33.1.34.48.72.36

2. COMPOSITION / INFORMATION ON INGREDIENTS

Chemical compounds	CAS N°	% or weight	Classification	LD50 (oral) (rat)
H Thrombin	NA	< 10%	NA	NA
Glycine	56-40-6	< 50%	022-24/25	7.93g/kg
Sodium chloride	7647-14-5	< 20%	024/25	3g/Kg
PEG 6000	25322-68-3	< 50%	R22-024/25	4.7 g/Kg
Calcium gluconate	299-28-5	< 50%	022-024/25	> 5g/kg
Hepes	7365-45-9	< 20%	NA	2g/kg
Ciprofloxacin	85721-33-1	< 10%	NA	NA
BSA	NA	< 30%	NA	NA

3. HEALTH HAZARDS IDENTIFICATION

All the above listed chemicals or biologicals may be harmful by inhalation, ingestion, or skin adsorption. Nasal irritation, eye reddening, and allergic reactions may result from overexposure.

Viral Safety: Purified human factors are extracted from human plasma, which has been tested for HIV Antibodies, HBs:Ag and HCV Antibodies, with registered methods. BSA and purified bovine factors were prepared from bovine plasma; which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no biological test may totally exclude the presence of any infectious agent. All biological material should be treated as potentially hazardous and the appropriate handling and disposal procedures must be adhered to.

4. FIRST AID MEASURES

-If swallowed, wash out mouth with water provided person is conscious. Medical advice is necessary.
-In case of contact with eyes, flush with copious amounts of water, for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Seek medical advice.
-In case of inhalation, remove victim to fresh air, and seek medical advice.
-In any case of overexposure, call a physician.
-In case of contact, inhalation or ingestion of any of the components of the kit, seek immediately medical attention. If the victim is conscious, in case of ingestion, drink several glasses of water (or milk), to dilute contents of stomach. Do not induce vomiting.

5. FIRE AND EXPLOSION HAZARDS DATA

Flammability: Only carton boxes, dry chemical, interiors inserts are flammable.

Extinguishing media: carbon dioxide, dry chemical powder or appropriate foam.

Special fire fighting procedures: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin.

6. SPILL, LEAK AND DISPOSAL PROCEDURES

Sweep up, place in a bag and hold for waste material. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete. Do not empty into drains or sinks. Comply with all federal, state and local environmental regulations on waste handling and disposal

7. CAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Must be used only by suitable trained and informed personnel
Wear chemical resistant gloves, chemical safety goggles and protective laboratory clothing.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION**Personal Protective Equipment**

Eyes: Wear chemical splash goggles
Skin: Wear appropriate protective gloves to prevent skin exposure
Clothing: Wear appropriate protective clothing to prevent skin exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Reagents are lyophilized powder. They do not present any specific physical or chemical reactivity, and are stable compounds.

10. STABILITY AND REACTIVITY

Stability: Stable

Hazardous combustion or decomposition products: Carbon monoxide, carbon dioxide, nitrogen oxide.

Hazardous polymerisation: Does not occur.

11. TOXICOLOGICAL INFORMATION

All the components of the kit are intended for in vitro use only, by experienced and suitably trained personal. There is no special risk when used in these conditions. Products may be toxic following skin or eye contact, inhalation or ingestion. For toxicity of components, refer to chapters 2 and 3.

12. ECOLOGICAL INFORMATION

Do not empty reagents into waters or drains. Comply with state and local environmental regulations. Usually wasted biological material is stocked in hermetic specific containers for incineration by specialized companies.

13. WASTE DISPOSAL CONSIDERATIONS

Any waste product or reagent must be discarded according to local considerations.

Do not reuse vials or containers.

"Biohazard" risk is mentioned on the box.

14. TRANSPORT AND STORAGE INFORMATION

The reagent must be shipped adequately packaged and protected from any break during transportation.

It can be shipped at ambient temperature for a short period, not exceeding 7 days. It must be stored in a cold room at 2-8°C upon receipt.

No special regulation for transporting this product.

General rules for in vitro use should apply.

Local, State and Federal regulations for this kind of product must be respected.

The kits must be stored in an appropriate refrigerated area, specifically dedicated for in vitro use kits.

All the storage constraints are indicated on the package labels and on the kits insert.

Product name: **Human Thrombin**

15. REGULATORY INFORMATION

This reagent is designed, manufactured, controlled and followed according to the quality management system (based on ISO 9001 and ISO 13485) developed by HYPHEN BioMed.

Risk analysis has been performed and reduced to the lowest level available from the present knowledge.

16. OTHER INFORMATION

For in vitro research use only.

Last revision date: 02/07/2009

Revision were made in sections: All

The information reported on this MSDS is believed to be accurate and represents the best information available to us. However, we make no warranty of merchantability or any other warranty, expressed or implied, with respect to such information, and we assure no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. HYPHEN BioMed and its appointed agents/distributors or OEM contractors shall not be held liable for any damage resulting from or from contact with the products included in the kit.

16.1.3 Calcium Chloride

Material Safety Data Sheet

Calcium Chloride, Anhydrous, 95%, Irregular Granules

ACC# 95782

Section 1 - Chemical Product and Company Identification

MSDS Name: Calcium Chloride, Anhydrous, 95%, Irregular Granules**Catalog Numbers:** AC219170010, AC219170025, AC219170250, AC219175000, AC300380000, AC300380010 AC300380010, AC300380025, AC300382500**Synonyms:** Calpus; Caltac; Dowflake; Liquidow; Peladow; Snowmelt; Superflake Anhydrous.**Company Identification:**

Acros Organics N.V.

One Reagent Lane

Fair Lawn, NJ 07410

For information in North America, call: 800-ACROS-01**For emergencies in the US, call CHEMTREC:** 800-424-9300

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
10043-52-4	Calcium chloride	95%	233-140-8

Hazard Symbols: XI**Risk Phrases:** 36

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: white solid. May be harmful if swallowed. May cause severe respiratory and digestive tract irritation with possible burns. May cause severe eye and skin irritation with

possible burns. May cause cardiac disturbances. Hygroscopic (absorbs moisture from the air).

Warning!

Target Organs: Heart.

Potential Health Effects

Eye: Contact with eyes may cause severe irritation, and possible eye burns.

Skin: Contact with skin causes irritation and possible burns, especially if the skin is wet or moist.

Ingestion: May cause severe gastrointestinal tract irritation with nausea, vomiting and possible burns. May cause cardiac disturbances. May be harmful if swallowed. In very severe cases, seizures, rapid respiration, slow heartbeat, or death, may result.

Inhalation: May cause severe irritation of the upper respiratory tract with pain, burns, and inflammation.

Chronic: Effects may be delayed.

Section 4 - First Aid Measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.

Skin: Get medical aid. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse.

Ingestion: Do NOT induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.

Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid. Do NOT use mouth-to-mouth resuscitation.

Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: Wear appropriate protective clothing to prevent contact with skin and eyes. Wear a self-contained breathing apparatus (SCBA) to prevent contact with thermal decomposition products.

Extinguishing Media: Use extinguishing media most appropriate for the surrounding fire.

Flash Point: Not applicable.

Autoignition Temperature: Not applicable.

Explosion Limits, Lower:Not available.

Upper: Not available.

NFPA Rating: (estimated) Health: 2; Flammability: 0; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks: Vacuum or sweep up material and place into a suitable disposal container. Clean up spills immediately, observing precautions in the Protective Equipment section. Avoid generating dusty conditions. Provide ventilation.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Use with adequate ventilation. Minimize dust generation and accumulation. Keep container tightly closed. Do not get on skin or in eyes. Do not ingest or inhale. Wash clothing before reuse. Always use cool water when dissolving Calcium Chloride. Heat evolved is significant.

Storage: Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances. Store protected from moisture. Store below melting point.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.

Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Calcium chloride	none listed	none listed	none listed

OSHA Vacated PELs: Calcium chloride: No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment

Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by

OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin: Wear impervious gloves.

Clothing: Wear appropriate protective clothing to minimize contact with skin.

Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Always use a NIOSH or European Standard EN 149 approved respirator when necessary.

Section 9 - Physical and Chemical Properties

Physical State: Solid

Appearance: white

Odor: odorless

pH: Not available.

Vapor Pressure: Not available.

Vapor Density: Not available.

Evaporation Rate:Not available.

Viscosity: Not available.

Boiling Point: > 1600 deg C @ 760.00mm Hg

Freezing/Melting Point:782 deg C

Decomposition Temperature:Not available.

Solubility: freely soluble in alcohol

Specific Gravity/Density:2.1500g/cm³

Molecular Formula:CaCl₂

Molecular Weight:110.99

Section 10 - Stability and Reactivity

Chemical Stability: Stable.

Conditions to Avoid: Dust generation, excess heat, exposure to moist air or water.

Incompatibilities with Other Materials: Bromine trifluoride, 2-Furanpercarboxylic Acid, Solutions attack some metals..

Hazardous Decomposition Products: Hydrogen chloride, calcium oxide.

Hazardous Polymerization: Has not been reported.

Section 11 - Toxicological Information

RTECS#:

CAS# 10043-52-4: EV9800000

LD50/LC50:

CAS# 10043-52-4:

Oral, mouse: LD50 = 1940 mg/kg;

Oral, rabbit: LD50 = 1384 mg/kg;

Oral, rat: LD50 = 1 gm/kg;<br.

Carcinogenicity:

CAS# 10043-52-4: Not listed by ACGIH, IARC, NIOSH, NTP, or OSHA.

Epidemiology: No information found.**Teratogenicity:** No information found.**Reproductive Effects:** No information found.**Neurotoxicity:** No information found.**Mutagenicity:** Mutagenic effects have occurred in experimental animals.**Other Studies:** See actual entry in RTECS for complete information. </br.

Section 12 - Ecological Information

No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.

RCRA P-Series: None listed.**RCRA U-Series:** None listed.

Section 14 - Transport Information

	US DOT	IATA	RID/ADR	IMO	Canada TDG
Shipping Name:	No information				No information

	available.				available.
Hazard Class:					
UN Number:					
Packing Group:					

Section 15 - Regulatory Information

US FEDERAL

TSCA

CAS# 10043-52-4 is listed on the TSCA inventory.

Health & Safety Reporting List

None of the chemicals are on the Health & Safety Reporting List.

Chemical Test Rules

None of the chemicals in this product are under a Chemical Test Rule.

Section 12b

None of the chemicals are listed under TSCA Section 12b.

TSCA Significant New Use Rule

None of the chemicals in this material have a SNUR under TSCA.

SARA

CERCLA Hazardous Substances and corresponding RQs

None of the chemicals in this material have an RQ.

SARA Section 302 Extremely Hazardous Substances

None of the chemicals in this product have a TPQ.

SARA Codes

CAS # 10043-52-4: acute, chronic, reactive.

Section 313

No chemicals are reportable under Section 313.

Clean Air Act:

This material does not contain any hazardous air pollutants. This material does not contain any Class 1 Ozone depletors. This material does not contain any Class 2 Ozone depletors.

Clean Water Act:

None of the chemicals in this product are listed as Hazardous Substances under the CWA. None of the chemicals in this product are listed as Priority Pollutants under the CWA. None of the chemicals in this product are listed as Toxic Pollutants under the CWA.

OSHA:

None of the chemicals in this product are considered highly hazardous by OSHA.

STATE

CAS# 10043-52-4 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations**European Labeling in Accordance with EC Directives****Hazard Symbols:**

XI

Risk Phrases:

R 36 Irritating to eyes.

Safety Phrases:

S 22 Do not breathe dust.

S 24 Avoid contact with skin.

WGK (Water Danger/Protection)

CAS# 10043-52-4: 0

Canada - DSL/NDSL

CAS# 10043-52-4 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of D2B.

Canadian Ingredient Disclosure List**Exposure Limits****Section 16 - Additional Information**

MSDS Creation Date: 6/29/1998

Revision #5 Date: 11/19/2001

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

16.1.4 Phosphate Buffered Solution (PBS)

Material Safety Data Sheet

PBS Phosphate Buffered Saline

ACC# 89342

Section 1 - Chemical Product and Company Identification

MSDS Name: PBS Phosphate Buffered Saline**Catalog Numbers:** BP661-10, BP661-50, BP665-1**Synonyms:** None.**Company Identification:**

Fisher Scientific

1 Reagent Lane

Fair Lawn, NJ 07410

For information, call: 201-796-7100**Emergency Number:** 201-796-7100**For CHEMTREC assistance, call:** 800-424-9300**For International CHEMTREC assistance, call:** 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
7647-14-5	Sodium Chloride	81.0	231-598-3
7558-79-4	Sodium phosphate, dibasic	~14	231-448-7
7778-77-0	Potassium phosphate, Monobasic	~3.0	231-913-4
7447-40-7	Potassium chloride	~2.0	231-211-8

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: white solid.

Warning! Causes eye irritation. May cause skin and respiratory tract irritation.

Target Organs: Eyes.

Potential Health Effects

Eye: Causes eye irritation.

Skin: May cause skin irritation.

Ingestion: Ingestion of large amounts may cause gastrointestinal irritation. May cause gastric disturbances and electrolytic imbalance. Ingestion of large amounts of sodium chloride may cause nausea, and vomiting, rigidity or convulsions. Continued exposure can produce coma, dehydration and internal organ congestion.

Inhalation: Inhalation of dust may cause respiratory tract irritation.

Chronic: No information found.

Section 4 - First Aid Measures

Eyes: Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.

Skin: Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or persists. Wash clothing before reuse.

Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.

Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.

Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion. Substance is noncombustible.

Extinguishing Media: Use water spray, dry chemical, carbon dioxide, or chemical foam.

Flash Point: Not applicable.

Autoignition Temperature: Not applicable.

Explosion Limits, Lower:Not available.

Upper: Not available.

NFPA Rating: (estimated) Health: 2; Flammability: 0; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks: Vacuum or sweep up material and place into a suitable disposal container. Clean up spills immediately, observing precautions in the Protective Equipment section. Avoid generating dusty conditions. Provide ventilation.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Use with adequate ventilation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation.

Storage: Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.

Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Sodium Chloride	none listed	none listed	none listed
Sodium phosphate, dibasic	none listed	none listed	none listed
Potassium phosphate, Monobasic	none listed	none listed	none listed

Potassium chloride	none listed	none listed	none listed
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OSHA Vacated PELs: Sodium Chloride: No OSHA Vacated PELs are listed for this chemical. Sodium phosphate, dibasic: No OSHA Vacated PELs are listed for this chemical. Potassium phosphate, Monobasic: No OSHA Vacated PELs are listed for this chemical. Potassium chloride: No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment

Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin: Wear appropriate protective gloves to prevent skin exposure.

Clothing: Wear appropriate protective clothing to prevent skin exposure.

Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: Solid

Appearance: white

Odor: none reported

pH: Not available.

Vapor Pressure: Not available.

Vapor Density: Not available.

Evaporation Rate:Not available.

Viscosity: Not available.

Boiling Point: Not available.

Freezing/Melting Point:Not available.

Decomposition Temperature:Not available.

Solubility: Soluble.

Specific Gravity/Density:Not available.

Molecular Formula:Mixture

Molecular Weight:Not available.

Section 10 - Stability and Reactivity

Chemical Stability: Stable.

Conditions to Avoid: Dust generation, excess heat.

Incompatibilities with Other Materials: Potassium chloride is incompatible with potassium permanganate. Sodium chloride is incompatible with dichloromaleic anhydride + urea, lithium, and nitrogen compounds. Potassium phosphate dibasic and monobasic may react violently with strong acids.

Hazardous Decomposition Products: Oxides of phosphorus, sodium oxide, oxides of potassium.

Hazardous Polymerization: Will not occur.

Section 11 - Toxicological Information

RTECS#:

CAS# 7647-14-5: VZ4725000

CAS# 7558-79-4: WC4500000

CAS# 7778-77-0: TC6615500

CAS# 7447-40-7: TS8050000

LD50/LC50:

CAS# 7647-14-5:

- Draize test, rabbit, eye: 100 mg Mild;
- Draize test, rabbit, eye: 100 mg/24H Moderate;
- Draize test, rabbit, eye: 10 mg Moderate;
- Draize test, rabbit, skin: 50 mg/24H Mild;
- Draize test, rabbit, skin: 500 mg/24H Mild;
- Inhalation, rat: LC50 = >42 gm/m³/1H;
- Oral, mouse: LD50 = 4 gm/kg;
- Oral, rat: LD50 = 3000 mg/kg;
- Skin, rabbit: LD50 = >10 gm/kg;

CAS# 7558-79-4:

- Draize test, rabbit, eye: 500 mg/24H Mild;
- Draize test, rabbit, skin: 500 mg/24H Mild;
- Oral, rat: LD50 = 17 gm/kg;

CAS# 7778-77-0:

- Skin, rabbit: LD50 = >4640 mg/kg;

CAS# 7447-40-7:

- Draize test, rabbit, eye: 500 mg/24H Mild;
- Oral, mouse: LD50 = 1500 mg/kg;
- Oral, rat: LD50 = 2600 mg/kg;

Carcinogenicity:

CAS# 7647-14-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

CAS# 7558-79-4: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

CAS# 7778-77-0: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

CAS# 7447-40-7: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No information found**Teratogenicity:** No information found**Reproductive Effects:** No information found**Mutagenicity:** No information found**Neurotoxicity:** No information found**Other Studies:**

Section 12 - Ecological Information

No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.

RCRA P-Series: None listed.**RCRA U-Series:** None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	Not regulated as a hazardous material	No information available.
Hazard Class:		
UN Number:		

Packing Group:		
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Section 15 - Regulatory Information

US FEDERAL

TSCA

CAS# 7647-14-5 is listed on the TSCA inventory.

CAS# 7558-79-4 is listed on the TSCA inventory.

CAS# 7778-77-0 is listed on the TSCA inventory.

CAS# 7447-40-7 is listed on the TSCA inventory.

Health & Safety Reporting List

None of the chemicals are on the Health & Safety Reporting List.

Chemical Test Rules

None of the chemicals in this product are under a Chemical Test Rule.

Section 12b

None of the chemicals are listed under TSCA Section 12b.

TSCA Significant New Use Rule

None of the chemicals in this material have a SNUR under TSCA.

CERCLA Hazardous Substances and corresponding RQs

CAS# 7558-79-4: 5000 lb final RQ; 2270 kg final RQ

SARA Section 302 Extremely Hazardous Substances

None of the chemicals in this product have a TPQ.

SARA Codes

CAS # 7647-14-5: immediate.

CAS # 7778-77-0: immediate.

CAS # 7447-40-7: immediate.

Section 313

This material contains Potassium chloride (listed as Water Dissociable Nitrate Compounds), ~2.0%, (CAS# 7447-40-7) which is subject to the reporting requirements of Section 313 of SARA Title III and 40 CFR Part 373.

Clean Air Act:

This material does not contain any hazardous air pollutants.

This material does not contain any Class 1 Ozone depleters.

This material does not contain any Class 2 Ozone depleters.

Clean Water Act:

CAS# 7558-79-4 is listed as a Hazardous Substance under the CWA.

None of the chemicals in this product are listed as Priority Pollutants under the CWA.

None of the chemicals in this product are listed as Toxic Pollutants under the CWA.

OSHA:

None of the chemicals in this product are considered highly hazardous by OSHA.

STATE

CAS# 7647-14-5 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

CAS# 7558-79-4 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Massachusetts.

CAS# 7778-77-0 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

CAS# 7447-40-7 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations**European Labeling in Accordance with EC Directives****Hazard Symbols:**

Not available.

Risk Phrases:**Safety Phrases:****WGK (Water Danger/Protection)**

CAS# 7647-14-5: 0

CAS# 7558-79-4: 1

CAS# 7778-77-0: 1

CAS# 7447-40-7: 1

Canada - DSL/NDSL

CAS# 7647-14-5 is listed on Canada's DSL List.

CAS# 7558-79-4 is listed on Canada's DSL List.

CAS# 7778-77-0 is listed on Canada's DSL List.

CAS# 7447-40-7 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of D2B.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

Canadian Ingredient Disclosure List

CAS# 7447-40-7 is not listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 7/16/1999

Revision #6 Date: 6/29/2007

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from

its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

16.1.5 Tris-Buffered Solution (TBS)

Close This Browser Window to Return to Data Sheet Index

MATERIAL SAFETY DATA SHEET

Effective Date: July 23, 2010

SECTION 1 PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME:	para-tertiary-Butylstyrene	CHEMICAL SYNONYMS:	1-(1,1-Dimethylethyl)-4-ethenylbenzene
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PRODUCT CODE:	TBS	CHEMICAL FAMILY:	Aromatic Hydrocarbon
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PRODUCT USE: Specialty Monomer

COMPANY NAME:	Deltech Corporation
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11911 Scenic
HighwayBaton Rouge, LA
70807

24 Hour EMERGENCY PHONE	(01) 225 772-0150	CHEMTREC:	1-800 424-9300
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SECTION 2 COMPOSITION/INFORMATION ON INGREDIENTS

Component (CAS #)	% By Weight	Exposure Limits
p-tert-Butylstyrene (1746-23-2)	94-96	Not Established
tert-Butylethylbenzene (37871-12-8)	2- 3	Not Established
m-tert-Butylstyrene (19789-36-7)	1- 3	Not Established

This material is Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).

SECTION 3

HAZARD IDENTIFICATION

EMERGENCY OVERVIEW

TBS is water-white liquid with aromatic odor. TBS is a moderate eye, respiratory system and skin irritant. TBS may polymerize and autoaccelerate if exposed to heat or mixed with catalyst, peroxide, metal halide, acids, or oxidizing agents resulting in rapid temperature rise and increased volatilization. Combustible. Hot vapors are extremely flammable and are heavier than air.

POTENTIAL HEALTH EFFECTS:

EYE: Vapors may cause irritation. Liquid is strong eye irritant.

SKIN: Exposure may cause redness and irritation of the skin. May cause drying and flaking of the skin. A single, prolonged exposure is not likely to result in the material being absorbed through the skin in harmful amounts.

INGESTION: Substance may be harmful if swallowed, although it is unlikely to have serious health effects in minute quantities. May cause central nervous system depression (dizziness, drowsiness), nausea and loss of consciousness.

INHALATION: Avoid breathing vapors or mists, may be harmful if inhaled. Because of low vapor pressure it is unlikely that TBS will be inhaled in harmful amounts. If heated or misted, concentrations may be attained that may be hazardous from a single exposure.

Irritation to the upper respiratory tract and lungs, central nervous system depression (dizziness, drowsiness), nausea and loss of consciousness are common consequences of inhalation overexposure

CHRONIC (CANCER) INFORMATION: No Data.

TERATOLOGY (BIRTH DEFECT) INFORMATION: No Data.

REPRODUCTION INFORMATION: No Data.

POTENTIAL ENVIRONMENTAL EFFECTS: No Data. The low volatility should significantly limit persistence and mitigate the potential to produce adverse environmental impacts.

SECTION 4

FIRST AID MEASURES

EYES: Flush eyes thoroughly with water for 15 minutes. Consult physician if irritation persists.

SKIN: Wash affected area with soap and water. Remove contaminated clothing and shoes. Do not reuse contaminated clothing without laundering. Consult physician if irritation persists.

INGESTION: Do not induce vomiting; consult physician immediately.

INHALATION: Remove victim to fresh air. Get medical assistance immediately. May cause chemical pneumonia if aspirated. Administer oxygen if there is difficulty breathing. Administer artificial respiration if not breathing.

NOTE TO PHYSICIANS

Because rapid absorption may occur through the lungs if aspirated, the decision of whether to induce vomiting should be made by a physician. If lavage is performed, suggest endotracheal and/or esophageal control. Danger from lung aspiration must be weighed against toxicity when considering emptying the stomach. Treat burns as thermal burns. Treatment based upon judgement of the physician in response to the reactions of the patient.

SECTION 5

FIRE FIGHTING MEASURES

FLAMMABLE PROPERTIES: Flash Point: 177 °F Method: TCC

Flammable. Hot vapors are heavier than air and very flammable. Vapors may travel considerable distances to ignition source and cause flash fires or explosions.

FLAMMABILITY LIMITS: (212 °F) Lower Flammability Limit: 1.0%
Upper Flammability Limit: 2.7%

AUTOIGNITION TEMPERATURE: 813°F (434°C)

HAZARDOUS COMBUSTION PRODUCTS: Carbon dioxide, carbon monoxide, and other toxic fumes of incomplete combustion. During a fire, smoke may contain mists of the original material.

EXTINGUISHING MEDIA: Carbon dioxide, foam or dry chemical. Water fog or fine spray; water may be ineffective. General purpose synthetic foams or protein foams are preferred.

FIRE FIGHTING INSTRUCTIONS: Use water spray to cool fire exposed containers, protect personnel, and disperse vapors and spills. Dike and collect water due to potential environmental damage and spread of fire with product carried across water surface. Use self-contained breathing apparatus and fight fire from safe distance due to explosion potential.

UNUSUAL HAZARDS ASSOCIATED WITH FIRE: Closed containers of TBS may build up explosive pressures when exposed to the heat of fires. Closed containers of TBS exposed to the heat of fires may begin to polymerize in an exothermic manner leading to autoacceleration and rapid pressure increase and explosion potential.

SECTION 6

ACCIDENTAL RELEASE MEASURES

SMALL SPILL: Absorb spill with an inert material (dry sand) and place in chemical waste container for disposal (see section 13). Do not use reactive absorbents.

LARGE SPILL(on land): Remove all sparking devices and ignition sources. Contain spilled liquid with dikes of earth. Pump water into diked area and collect product from the

top of water. Dispose of hydrocarbon laden water accordingly. Use oil spill collection pads and booms to contain runoff and seepage from diked areas.

SPILLS INTO WATERWAYS: Contain spill with oil booms and recover product by vacuum truck or oil collection pads.

REPORTABLE QUANTITY: Not a DOT listed Hazardous Substance. Various state and local regulations may apply.

SECTION 7

HANDLING AND STORAGE

HANDLING: Avoid contact with eyes. Avoid prolonged or repeated contact with skin. Keep containers tightly closed and use in well ventilated areas. Avoid prolonged or repeated breathing of vapors. Use grounding and bonding connections when transferring material to prevent static discharge, fire or explosion. Use spark proof tools and explosion proof equipment. Even empty containers may contain vapors. Do not cut, drill, grind or weld on containers, even if emptied of product.

STORAGE: Store in a cool area or refrigerated tank away from high temperatures, hot pipes or direct sunlight. Maintain TBS temperature in storage below 90°F (32°C). Maintain inhibitor concentration above 50 ppm. If storage of more than 6 weeks is required aerate once per week with dry air to maintain dissolved oxygen above 10 ppm.

SECTION 8

EXPOSURE CONTROLS / PERSONAL PROTECTION

ENGINEERING CONTROLS: Use local ventilation to maintain airborne concentrations below exposure limits. Use only with adequate ventilation.

RESPIRATORY PROTECTION: For operations where inhalation exposure may occur, a NIOSH approved air purifying respirator with organic vapor cartridge(s) or canister may be permissible. Protection provided by air purifying respirators is limited. Use a positive-pressure air-supplied respirator if there is any potential for uncontrolled release or any other circumstances where air-purifying respirators may not provide adequate protection.

SKIN PROTECTION: When contact may occur, use protective clothing and gloves impervious to hydrocarbon materials. Use of specific items such as face shield, apron, gloves, boots or body suit is dependent upon operation. Wash hands thoroughly before

eating, drinking or smoking.

EYE PROTECTION: Use safety glasses when handling small amounts. When splashing may occur use chemical splash goggles and face shield. If vapors cause eye discomfort use a full-face, supplied-air respirator.

SECTION 9**PHYSICAL AND CHEMICAL PROPERTIES**

BOILING POINT	426°F (219°C)
MELTING POINT:	-36°F (-38°C)
VAPOR PRESSURE:	0.18 mmHG @68°F (20°C)
VAPOR DENSITY:	5.55 (air=1)
SOLUBILITY IN WATER:	Insoluble (5 ppm)
DENSITY:	0.884 g/cc @ 77°F (25°C)
pH:	N/A
ODOR:	Strong Aromatic Hydrocarbon
APPEARANCE:	Colorless Liquid

SECTION 10**STABILITY AND REACTIVITY**

CHEMICAL STABILITY: Stable under recommended storage conditions. Inhibited with tertiary-butyl-catechol (TBC). Maintain temperature below 90°F (32°C). Maintain oxygen content above 10 ppm.

CONDITIONS TO AVOID: Do not blanket with inert gas to avoid depleting dissolved oxygen concentration. Avoid excessive heat and keep away from open flames or ignition sources. Avoid dead-headed pumps while transferring.

INCOMPATIBILITY: Do not use copper or brass tubing or connections. Do not mix with oxidizing agents, acids, metal halides or peroxides.

HAZARDOUS DECOMPOSITION PRODUCTS : None known.

HAZARDOUS POLYMERIZATION: Polymerization may occur if exposed to excessive heat or catalyzed by mixture with incompatible materials. Polymerization is exothermic and may result in autoacceleration and increased pressure, venting of container, and a fire or explosion hazard.

SECTION 11**TOXICOLOGICAL INFORMATION**

EYE EFFECTS: No Data. Would be expected to be a mild to moderate eye irritant with toxicity resembling that of styrene.

SKIN EFFECTS: No Data. Would be expected to be a mild to moderate skin irritant with toxicity resembling that of styrene.

ACUTE ORAL EFFECTS: Oral LD50 in rats is >2000mg/kg.

ACUTE INHALATION EFFECTS: No Data. Would be expected to be a mild to moderate respiratory irritant with toxicity resembling that of styrene.

SUBCHRONIC EFFECTS: No Data.

CHRONIC EFFECTS / CARCINOGENICITY: No Data.

TERATOLOGY: No Data.

REPRODUCTION: No Data.

MUTAGENICITY: No Data.

SECTION 12**ECOLOGICAL INFORMATION**

ECOTOXICITY: The 96 hour static LC50 for fathead minnows is ~2 mg/L. The 48 hour static LC50 for *Daphnia magna* is ~1 mg/L. In each case the “No observed adverse effect” level was approximately 50% of LC50.

CHEMICAL FATE INFORMATION: Biodegradation under aerobic conditions is expected to be low, however the material is expected to biodegrade in a wastewater treatment plant. TBS is not expected to bioconcentrate in aquatic systems.

SECTION 13**DISPOSAL CONSIDERATIONS**

DISPOSAL: Do not dump into sewers, on the ground, into any body of water or into municipal or industrial waste receptacles. Disposal must be in accordance with all local, state and Federal/Provincial laws and regulations governing the disposal of chemical wastes. The preferred method of disposal for unusable or contaminated product is sending to a licensed, permitted incinerator or thermal destruction device. The heat of combustion of the material in the product form is approximately 18,000 BTUs/lb.

RECYCLING OPPORTUNITIES: Downgraded or unused product could possibly be returned to Deltech for recycling if certain criteria are met. Contact a Deltech sales representative for more information.

SECTION 14**TRANSPORT INFORMATION**

PROPER SHIPPING NAME Combustible Liquid, N.O.S.
(Para-Tertiary-Butyl Styrene, Stabilized)
NA 1993, PGIII

OTHER DOT REQUIREMENTS Not regulated in drum quantities (non-bulk)

SECTION 15**REGULATORY INFORMATION**

Not meant to be all inclusive.

US FEDERAL / STATE REGULATIONS:

CERCLA: SARA TITLE III SECTIONS 311 AND 312 HAZARD CATEGORY:
Acute Health Hazard

Fire
Chronic Health Hazard

Hazard

This notice must not be detached from the MSDS.

TSCA: Listed

PENNSYLVANIA RIGHT-TO-KNOW HAZARDOUS SUBSTANCES (34 Pa. Code Chap. 301-323) (Threshold 1%): Listed

INTERNATIONAL REGULATIONS:

CANADIAN DSL INVENTORY: Listed

EINCS: This product is on the European Inventory of Existing Commercial Chemical Substances. EINECS Inventory Number: 2171269

SECTION 16

OTHER INFORMATION

MSDS STATUS:

All sections revised to bring MSDS in compliance with the ANSI Z400.1-1998 Standard (10/02).

NATIONAL FIRE PROTECTION ASSOCIATION (NFPA) SYSTEM RATING:

The NFPA system rating for this product is: Fire - 2 Health - 2 Reactivity - 2

This material may be regulated by Louisiana's Right-To-Know Law, R.S.30:2361 ET SEQ.

Deltech believes the information and recommendations contained herein to be accurate and reliable. Since the foregoing is provided without charge and since use conditions and disposal are not within its control, Deltech assumes no obligation or liability for such information and recommendations and does not guarantee results from use of products described or other information contained herein. Deltech makes no warranties, express or implied, including merchantability or fitness for purpose; nor is freedom from any patent owned by Deltech or others to be inferred; and Deltech disclaims all liability for any resulting loss or damage.

16.1.6 Cell Lysis Buffer

#9803 MSDS

Material Safety Data Sheet (MSDS) for
Cell Lysis Buffer (10X)

Rev. 12/01/11

I. Identification:

Product name: Cell Lysis Buffer (10X)
Product Catalog: 9803
CAS number: Not applicable to mixtures
Manufacturer Supplier: Cell Signaling Technology
 3 Trask Lane
 Danvers, MA 01923 USA
 1-978-867-2300 TEL
 1-978-867-2400 FAX
 1-978-578-6737 Emergency Phone

II. Composition/Information on Ingredients:

This product is For Research Use Only. According to 29 CFR 1910.1200(d), mixtures with hazardous ingredients at less than <1% and carcinogens at less than <0.1% are considered non-hazardous.

Hazardous Reagent:

Ingredient	Percent	CAS#
Triton X-100 (polyethylene glycol octylphenol ether)	1%	9002-93-1

III. Hazard Identification:

!! CAUTION: This product is not for use in humans. It is intended for research purposes only. To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been established.

Emergency Overview :

Harmful by Ingestion. Irritant.

Potential Health Effects:

Inhalation: May be harmful if inhaled. Causes respiratory tract irritation.

Eye Contact: Causes eye irritation. Risk of damage to eyes.

Skin Contact: May be harmful if absorbed through skin. Causes skin irritation.

Ingestion: Harmful if swallowed.

IV. First Aid Measures for Hazardous Ingredient:**Triton X-100 (polyethylene glycol octylphenol ether):**

Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.

Ingestion: DO NOT INDUCE VOMITING. If person is conscious, wash out mouth with water. Get medical attention.

Skin contact: Wash skin with soap or mild detergent and water for at least 15 minutes. If irritation develops or persists, get medical attention.

Eye contact: Immediately flush eyes with water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: data not available

Autoignition Temperature: data not available

Explosion: data not available

Fire extinguishing media: water spray, dry chemical, foam, or carbon dioxide.

Firefighting: wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes. May emit toxic fumes under fire conditions.

VI. Accidental Release Measures:

Wear appropriate personal protective equipment as indicated in Section 8. Absorb liquid with an absorbent material. Transfer contaminated absorbent to a closed chemical waste container for disposal. Wash spill site after material has been picked up for disposal.

VII. Handling and Storage:

Store at 4°C in tightly closed container.

Avoid inhalation of vapor or mist. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Avoid prolonged or repeated exposure.

VIII. Exposure Controls/Personal

Ventilation System: a system of local (fume hood) and general exhaust is recommended.

Skin Protection: wear compatible chemical resistant gloves and protective clothing.

Eye protection: wear chemical safety goggles. Maintain emergency eyewash and shower in work area.

IX. Physical and Chemical Properties

Appearance: Colorless liquid
Odor: Data not available
pH: Data not available
Boiling Point: Data not available
Melting or Freezing Point: Data not available
Flash Point: Data not available
Volatile Organic Compounds (VOC): Data not available
Autoignition temp. Data not available
Solubility (water): Soluble in water

X. Stability and Reactivity:

Stability: Stable under ordinary conditions.

Conditions/materials to avoid: Strong oxidizing agents, strong acids, strong bases

Hazardous Decomposition: Carbon monoxide, carbon dioxide.

Hazardous polymerization: Will not occur.

XI. Toxicological Information:

Acute toxicity: Data not available

Chronic exposure: Data not available

Potential Health Effects:

Inhalation: May be harmful if inhaled. Causes respiratory tract irritation.

Skin: May be harmful if absorbed through skin. Causes skin irritation.

Eyes: Causes eye irritation.

Ingestion: Harmful if swallowed.

XII. Ecological Information:

Data not available

XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

XIV. Transport Information:

D.O.T. Proper Shipping Name: None. This substance is considered non-hazardous for transport. IATA Proper Shipping Name: None. This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

EU: Not classified

OSHA: Ingredient Triton X100, CAS#9002-93-1: Harmful by Ingestion, Irritant

Canadian DSL: Listed: Ingredient Triton X100, CAS#9002-93-1

SARA 302, 313 Ingredients Not Listed.

SARA 311/312: Ingredient Triton X100, CAS#9002-93-1: Acute Health Hazard

Massachusetts Right To Know: Ingredients Not Listed.

Pennsylvania Right To Know: Ingredient Triton X100, CAS#9002-93-1

New Jersey Right To Know: Ingredient Triton X100, CAS#9002-93-1

California Prop. 65: Ingredients Not Listed.

XVI. Other Information:

This product is for research use only and is not intended for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. The above information is believed to be accurate but is not necessarily all-inclusive and shall be used only as a guide. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.

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Orders ■ 877-616-CELL (2355) orders@cellsignal.com Support ■ 877-678-TECH (8324) info@cellsignal.com Web ■ www.cellsignal.com

16.1.7 Dynabeads



Material Safety Data Sheet

Revision Date: 26-Apr-2010

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 11368D FLOWCOMP DYNABEADS
 Product name FlowComp Dynabeads

Company/Undertaking Identification

INVITROGEN CORPORATON
 5791 VAN ALLEN WAY
 PO BOX 6482
 CARLSBAD, CA 92008
 760-603-7200

INVITROGEN CORPORATION
 5250 MAINWAY DRIVE
 BURLINGTON, ONT
 CANADA L7L 6A4
 800-263-6236

GIBCO PRODUCTS
 INVITROGEN CORPORATION
 3175 STALEY ROAD P.O. BOX 68
 GRAND ISLAND, NY 14072
 716-774-6700

24 hour Emergency Response 866-536-0631
 (Transport): 301-431-8585
 Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
sodium chloride	7647-14-5	0.1-1.0
Sodium Phosphate Buffer	None	0.1-1.0
Protein coupled dynabeads	Not available	1-5
BSA standard	NOT FOUND	0.1-1.0
Water, distilled, conductivity or of similar purity	7732-18-5	60-100
sodium azide	26628-22-8	<0.1

3. HAZARDS IDENTIFICATION

3. HAZARDS IDENTIFICATION

Emergency Overview
No information available

Form
Suspension

Principle Routes of Exposure/

Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects

No information available

HMIS

Health	No Information Available
Flammability	No Information Available
Reactivity	No Information Available

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
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7. HANDLING AND STORAGE

Storage Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
sodium chloride	-	-	-	-
Sodium Phosphate Buffer	-	-	-	-
Protein coupled dynabeads	-	-	-	-
BSA standard	-	-	-	-
Water, distilled, conductivity or of similar purity	-	-	-	-
sodium azide	-	-	-	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection In case of insufficient ventilation wear suitable respiratory equipment

Hand protection Protective gloves

Eye protection Safety glasses with side-shields

Skin and body protection Lightweight protective clothing.

Hygiene measures Handle in accordance with good industrial hygiene and safety practice

Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Suspension

Important Health Safety and Environmental Information

Boiling point/range °C No data available °F No data available

Melting point/range °C No data available °F No data available

Flash point °C No data available °F No data available

Autoignition temperature °C No data available °F No data available

Oxidizing properties No information available

Water solubility No data available

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.

Materials to avoid No information available

Hazardous decomposition products No information available

10. STABILITY AND REACTIVITY

Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION**Acute toxicity**

Chemical Name	LD50 (oral, rat/mouse)	LD50 (dermal, rat/rabbit)	LC50 (inhalation, rat/mouse)
sodium chloride	3 g/kg (Rat)	10 g/kg (Rabbit)	42 g/m ³ (Rat)
Sodium Phosphate Buffer	No data available	No data available	No data available
Protein coupled dynabeads	No data available	No data available	No data available
BSA standard	No data available	No data available	No data available
Water, distilled, conductivity or of similar purity	No data available	No data available	No data available
sodium azide	27 mg/kg (Rat)	20 mg/kg (Rabbit)	No data available

Principle Routes of Exposure/**Potential Health effects**

Eyes No information available
 Skin No information available
 Inhalation No information available
 Ingestion No information available

Specific effects

Carcinogenic effects No information available
 Mutagenic effects No information available
 Reproductive toxicity No information available
 Sensitization No information available

(Long Term Effects)

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects No information available.
 Mobility No information available.
 Biodegradation No information available.
 Bioaccumulation No information available

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION**IATA**

Proper shipping name Not classified as dangerous in the meaning of transport regulations
 Hazard Class No information available
 Subsidiary Class No information available
 Packing group No information available
 UN-No No information available

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
sodium chloride	Listed	Listed	Listed	Listed	-	Listed
Sodium Phosphate Buffer	-	-	-	-	-	-
Protein coupled dynabeads	-	-	-	-	-	-
BSA standard	-	-	-	-	-	-
Water, distilled, conductivity or of similar purity	Listed	Listed	-	Listed	-	Listed
sodium azide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313

This product is not regulated by SARA.

Chemical Name

sodium azide

CAS-No

28628-22-8

Weight %

<0.1

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)

This product does not contain HAPs.

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
sodium chloride	-	-	-	-	-
Sodium Phosphate Buffer	-	-	-	-	-
Protein coupled dynabeads	-	-	-	-	-
BSA standard	-	-	-	-	-
Water, distilled, conductivity or of similar purity	-	-	-	-	-
sodium azide	Listed	-	Listed	-	Listed

California Proposition 65

This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:

Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

For research use only

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

16.1.8 AntiCD45RA Antibody



Material Safety Data Sheet

Revision Date: 26-Apr-2010

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 11368D FLOWCOMP HUMAN CD45RA ANTIBODY
 Product name FLOWCOMP HUMAN CD45RA ANTIBODY

Company/Undertaking Identification

INVITROGEN CORPORATON
 5791 VAN ALLEN WAY
 PO BOX 6482
 CARLSBAD, CA 92008
 760-603-7200

INVITROGEN CORPORATION
 5250 MAINWAY DRIVE
 BURLINGTON, ONT
 CANADA L7L 6A4
 800-263-6236

GIBCO PRODUCTS
 INVITROGEN CORPORATION
 3175 STALEY ROAD P.O. BOX 68
 GRAND ISLAND, NY 14072
 716-774-6700

24 hour Emergency Response 866-536-0631
 (Transport): 301-431-8585
 Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
BSA standard	NOT FOUND	0.1-1.0
sodium azide	26628-22-8	<0.1

3. HAZARDS IDENTIFICATION

Emergency Overview
 No information available

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/Potential Health effects

Eyes	No information available.
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMS

Health	No Information Available
Flammability	No Information Available
Reactivity	No Information Available

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
BSA standard	-	-	-	-
sodium azide	-	-	-	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection In case of insufficient ventilation wear suitable respiratory equipment

Hand protection Protective gloves

Eye protection Safety glasses with side-shields

Skin and body protection Lightweight protective clothing.

Hygiene measures Handle in accordance with good industrial hygiene and safety practice

Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range °C No data available °F No data available

Melting point/range °C No data available °F No data available

Flash point °C No data available °F No data available

Autoignition temperature °C No data available °F No data available

Oxidizing properties No information available

Water solubility No data available

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.

Materials to avoid No information available

Hazardous decomposition products No information available

Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral, rat/mouse)	LD50 (dermal, rat/rabbit)	LC50 (inhalation, rat/mouse)
BSA standard	No data available	No data available	No data available
sodium azide	27 mg/kg (Rat)	20 mg/kg (Rabbit)	No data available

Principle Routes of Exposure/Potential Health effects

Eyes	No information available.
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

(Long Term Effects)Target Organ Effects

No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	No information available.
Bioaccumulation	No information available

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATIONIATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATIONInternational Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
BSA standard	-	-	-	-	-	-
sodium azide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal RegulationsSARA 313

This product is not regulated by SARA.

Chemical Name

sodium azide

CAS-No

28628-22-8

Weight %

<0.1

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)

This product does not contain HAPs.

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
BSA standard	-	-	-	-	-
sodium azide	Listed	-	Listed	-	Listed

California Proposition 65

This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:

Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

For research use only

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

16.2 Reagent Volume

16.2.1 Serum Filtration Calculations

5×10^{-4} mg of thrombin with an activity of 400 NIH units/mg is hydrated with 10 microL of TBS buffer to arrive at a concentration of .05mg thrombin/mL . This is added to 200 microL of plasma to arrive at a concentration of 1NIH unit/mL.

Assuming we need to neutralize it all citrate, it will take 34mg CaCl₂. If this is hydrated with 10microL TBS, you arrive at a concentration of 3.5M, which may not be possible. We can likely use less, probably ~5-15mg of CaCl₂.

Immunomagnetic Precipitation Calculations:

Per the manufacturer's protocol (attached next), 25 μ L of antibody is needed for 2 mL of whole blood used. However, the protocol has been modified to run under a shorter amount of time. Based on the antibody binding chart displayed in Chapter 7, we assumed a minimum of 50% antibody binding in 5 minutes. Therefore, for 1 mL of whole blood processed we assumed 25 μ L of antibody would be necessary. Conservatively accounting for losses in the wash steps, this number was doubled to 50 μ L. Likewise, the manufacturer's protocol calls for 75 μ L of beads per 2 mL of whole blood. Under the same assumptions, this number was doubled and then a small portion added to give 100 μ L for 1 mL of whole blood under the time-modified procedure used in our cartridge.



Dynabeads® FlowComp™ Human CD45RA

Isolation directly from buffy coat and whole blood

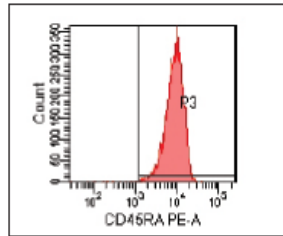
Catalog no. 11368D

Store at 2°C to 8°C

Rev. Date: February 2012 (Rev. 001)

Kit Contents

Kit contents	Volume
FlowComp™ Human CD45RA Antibody	1 mL
FlowComp™ Dynabeads®	3 mL
FlowComp™ Release Buffer	2 × 20 mL
Kit capacity	
Whole blood: 80 mL	

Figure 1: CD45RA⁺ cells after isolation.

FlowComp™ Dynabeads® contains ~1.5 × 10⁹ (~15 mg) beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. FlowComp™ Human CD45RA Antibody contains monoclonal CD45RA antibody in PBS with 0.5% BSA and 0.02% sodium azide. FlowComp™ Release Buffer contains modified biotin in 0.1% BSA and 2 mM EDTA. **Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

This product is intended for positive magnetic isolation of human CD45RA⁺ cells directly from anti-coagulated whole blood/buffy coat or from pre-selected CD4⁺ or CD8⁺ T cells. Thus no sample preparation (e.g. density gradient centrifugation or lysis of red blood cells) is required (fig. 1). For isolation from PBMC, see separate protocol supplied. In the first step, FlowComp™ Human CD45RA Antibody is added and binds to the target cells. In the second step, CD45RA⁺ T cells, that have bound

the specific antibodies, are captured by the FlowComp™ Dynabeads®. In the third and last step, the cells are released from the FlowComp™ Dynabeads® and can be used in any downstream applications.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Isolation Buffer: Ca²⁺ and Mg²⁺ free PBS supplemented with 0.1% BSA and 2 mM EDTA. **Note:** BSA can be replaced by human serum albumin (HSA) or 2% FBS/FCS.
- *Optional:* Flow cytometry antibodies. We recommend using mouse anti-human CD45RA (clone HI100) as primary fluorescent antibody for flow staining of cells after isolation.
- *Optional:* For viability analysis, SYTOX® Red is recommended.

General Guidelines

- Use a mixer that provides tilting and rotation of the tubes to ensure that beads do not settle in the tube.
- Avoid spilling of sample in tube cap during rotating and tilting. If so, change tube cap. We recommend raising one end of the rotator during incubation.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than the recommended volume of beads.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.
- To avoid unspecific labeling of cells during flow staining, we recommend using gammaglobulin prior to staining with primary fluorescent antibody.
- For better purity, repeat the washing step once or transfer the bead-bound cells to a new tube before adding the FlowComp™ Release Buffer.
- All incubations at room temperature can also be performed at 2°C to 8°C.

Protocol

This protocol is intended for isolation of bead-free CD14⁺ monocytes starting with 5 × 10⁷ PBMC/test where typically 10–20% are CD14⁺ monocytes. When working with higher cell numbers/number of tests, scale up all volumes accordingly, as shown in Table 1.

Wash the Beads

See Table 1 for volume recommendations.

1. Resuspend the beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of beads to a tube.
3. Add the same volume of Isolation Buffer from step 2, or at least 1 mL, and resuspend.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed beads in the same volume of Isolation Buffer as the initial volume of beads (step 2).

Prepare Cells

- Collect whole blood sample in a collection tube containing an appropriate anticoagulant, (e.g. EDTA, heparin, ACD or citrate).
- Prepare approximately 20 mL of Isolation Buffer per 2 mL whole blood.

Isolate Cells

This protocol is based on 2 mL whole blood/buffy coat, but is scalable according to Table 1.

1. Transfer 2 mL pre-cooled anti-coagulated whole blood/buffy coat to a tube on ice and add 25 μ L FlowComp™ Human CD45RA Antibody.
2. Mix well and incubate for 10 min on ice.
3. Add 4 mL Isolation Buffer and mix well, followed by centrifugation for 15 min at 350 \times g with no brakes.
4. Aspirate the supernatant and discard the 4 mL volume added in step 3 (but keep at least 1 cm above cell pellet to avoid losing leucocytes).
5. Add 75 μ L resuspended FlowComp™ Dynabeads® and mix well by vortexing.
6. Incubate for 15 min at room temperature under rolling and tilting.
7. Add 4 mL Isolation Buffer, mix well (or vortex 2–3 sec) and place the tube in the magnet for minimum 3 min.
8. While the tube is still in the magnet, carefully remove and discard the supernatant containing the CD45RA negative cells.
9. Repeat steps 7–8 twice to wash the bead-bound CD45RA⁺ cells. These steps are critical to obtain a high purity of isolated cells.

Release Cells

10. Resuspend in 1 mL FlowComp™ Release Buffer and pipet 3–4 times.
11. Incubate for 10 min at room temperature under rolling and tilting.
12. Pipet 10 times to efficiently release the cells and place in a magnet for 1 min. Avoid foaming.
13. Transfer the supernatant containing the bead-free cells to a new tube and again place on the magnet for 1 min to remove any residual beads. Transfer again the supernatant containing the bead-free cells to a new tube.
14. Add 2 mL Isolation Buffer followed by centrifugation for 8 min at 350 \times g. Discard the supernatant and resuspend the cell pellet in preferred cell medium.

Keep the cells on 2°C to 8°C until further use in downstream applications.

Table 1: Volumes for human CD45RA⁺ cells from whole blood/buffy coat. This protocol is scalable from 2–25 mL whole blood/buffy coat.

Step	Step description	Volumes per 2 mL blood/buffy	Volumes per 20 mL blood/buffy
	Recommended tube size	5 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Whole blood	2 mL	20 mL
1	FlowComp™ Human CD45RA Antibody	25 μ L	250 μ L
3	Wash cells (Isolation Buffer)	4 mL	40 mL
5*	FlowComp™ Dynabeads®	75 μ L	750 μ L
7–9	Wash beads (Isolation Buffer)	3 \times 4 mL	3 \times 40 mL
10	FlowComp™ Release Buffer	1 mL	10 mL
14	Wash cells (Isolation Buffer)	2 mL	20 mL

*When incubating, tilt and rotate the vial so the cells and beads are kept in the bottom of the tube. Do not perform end-over-end mixing if the volume is small relative to the tube size.

Description of Materials

FlowComp™ Dynabeads® are uniform, superparamagnetic polystyrene beads (2.8 μ m in diameter) coated with modified streptavidin. FlowComp™ Human CD45RA Antibody contains a DSB-X conjugated monoclonal mouse anti-human CD45RA. FlowComp™ Release Buffer contains a modified biotin that displaces the modified biotin on the antibody to release cells from the beads.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Mouse anti-human CD45RA	MHCD0804
SYTOX® Red	S34859

[REF] on labels is the symbol for catalog number.

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Manufactured by Life Technologies AS, Norway. Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support. 13485:2003.

SPEC-07232

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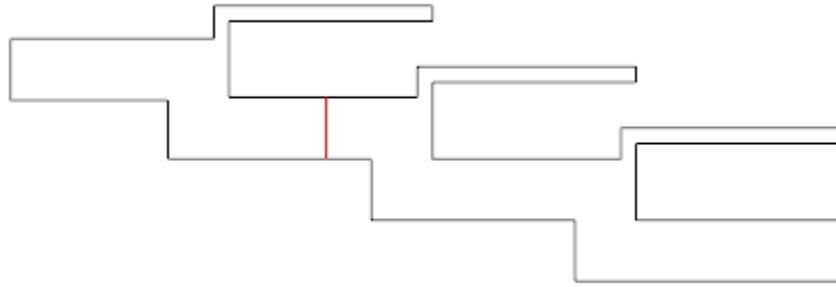
For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

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16.3 Channel Bifurcation Calculations

COMSOL was used to calculate the fluid mechanics involved with blood flowing through the microfluidic channels. This was done according to the procedure outlined in Equations 4.1 through 4.3. Specifically, the average velocities were calculated by creating cut lines as shown below:



COMSOL then reported an average velocity across the cutline. These values were used as outlined in Chapter 4 to calculate the flow rate ratios as in the example trials.

Since we were told to have a plasma output of $> 100 \mu\text{L}$ and a serum output of $> 100 \mu\text{L}$, this necessitated that the microfluidic device be capable of outputting at least $200 \mu\text{L}$ of relatively pure plasma in the allotted run time. Assuming a flowrate of $10.5 \mu\text{L}/\text{min}$ and, for the design parameters of a main channel width of $60 \mu\text{m}$ and daughter channel width of $15 \mu\text{m}$, the following calculations were used to determine total plasma output for a run time of 20 minutes:

$$A_{cross\ section} = 15 \mu\text{m} \cdot 35 \mu\text{m} = 5.25 \cdot 10^{-10} \text{m}^2 \quad (1)$$

Then, the flowrates through each of the daughter channels was calculated as:

$$Q_{daughter,i} = u_i \cdot A_{cross\ section} \quad (2)$$

The flow rates through each of the daughter channels were then added as follows:

$$Q_{daughter} = \sum_i u_i \cdot A_{cross\ section} = A_{cross\ section} \cdot \sum_i u_i = 4.87 \cdot 10^{-11} m^3/s \quad (3)$$

The total plasma volume is then given by:

$$V_{plasma} = 4\ channles \cdot Q_{daughter} \cdot t_{run} = 4 \cdot 4.87 \cdot 10^{-11} \left(\frac{m^3}{s}\right) \cdot 20\ min = 234.1\ \mu L(4)$$

Since this volume came from a flow rate of 10.5 $\mu L/min$, for 20 minutes, 840 μL of whole blood was required, indicating that for the first bifurcation process, half this amount is needed (420 μL indicating at least 105 μL are needed in each well). Conservatively, we placed 120 μL of whole blood in each well for each bifurcation step. The amount of plasma being taken from the whole blood is therefore 27.8% by volume, which seems reasonable given that blood is nearly 55% plasma by volume.

These calculations seemed adequate for the given design but can be modified during the trial year as necessary and according to experimental data.

16.4 Darcy's Law Calculation

$$Q = A \frac{k \Delta P}{\mu L}$$

$$\text{Area of membrane} = \pi r^2 = \pi (.694/2)^2 = 0.120409$$

Volume compressed fibrin

$$= \text{Volume of plasma} * \frac{[\text{fibrinogen}]}{\text{density of fibrin}} = 200 \mu\text{L plasma} * \frac{2.5\text{g}}{\text{L}} * \frac{5\text{mL}}{\text{g}} * \frac{.0001\text{L}}{\mu\text{L}} = .0025\text{mL}$$

compressed fibrin

$$\text{Height of fibrin gel} = V = \pi r^2 h, \quad h = \frac{V}{\pi r^2} = \frac{.0025}{\pi (.12)^2} = .0066$$

$$Q = A \frac{k \Delta P}{\mu L} = .1204 * \frac{10^{-13} \text{cm}^2/\text{s} \cdot 345000 \text{Pa}}{.001 \text{Pa} * \text{s} \cdot .0066} = 52 \mu\text{L}/\text{s}$$

Flow rate through membrane = 52 $\mu\text{L}/\text{s}$

16.5 Financial Appendix

16.5.1 Market Projections

Market Projections

Base Case

Assumptions Price of Cartridge: \$25
 Price of Processing Unit: \$100K
 50% penetration after 2 years of R&D trials
 Growth rate of t: 5.50%

	1	2	3	4	5	6	7	8	9	10	11
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Total Number of Clinical Sites	1000.00	1055.00	1113.03	1174.24	1238.82	1306.96	1378.84	1454.68	1534.69	1619.09	1708.14
Total Number of Blood Samples Processed	1,000,000	1,055,000	1,113,025	1,174,241	1,238,825	1,306,960	1,378,843	1,454,679	1,534,687	1,619,094	1,708,144
% of market share	0%	15%	30%	50%	50%	50%	50%	50%	50%	50%	50%
Total Number of Cartridge Sold	-	158,250	333,908	587,121	619,412	653,480	689,421	727,340	767,343	809,547	854,072
Price per Cartridge w/o Inflation	\$25.00	\$25.00	\$25.00	\$25.00	\$25.00	\$25.00	\$25.00	\$25.00	\$25.00	\$25.00	\$25.00
Inflation	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%
Price per Cartridge w/ Inflation	\$25.00	\$25.63	\$26.27	\$26.92	\$27.60	\$28.29	\$28.99	\$29.72	\$30.46	\$31.22	\$32.00
Total Cartridge Revenue	\$0	\$4,055,156	\$8,770,289	\$15,806,619	\$17,092,883	\$18,483,816	\$19,987,937	\$21,614,455	\$23,373,331	\$25,275,336	\$27,332,117
Total Number of Processing Units Sold	-	158	334	587	32	34	36	38	40	42	45
Price per Unit w/o Inflation	\$100,000.00	\$100,000.00	\$100,000.00	\$100,000.00	\$100,000.00	\$100,000.00	\$100,000.00	\$100,000.00	\$100,000.00	\$100,000.00	\$100,000.00
Inflation	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%
Price per Unit w/ Inflation	\$100,000.00	\$102,500.00	\$105,062.50	\$107,689.06	\$110,381.29	\$113,140.82	\$115,969.34	\$118,868.58	\$121,840.29	\$124,886.30	\$128,008.45
Total Processing Unit Revenue	\$0	\$16,220,625	\$35,081,157	\$63,226,476	\$3,564,393	\$3,854,445	\$4,168,101	\$4,507,280	\$4,874,060	\$5,270,686	\$5,699,588
% of Maintenance and Repair Services {{(Rev)}}	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%
Total Maintenance and Repair Services	\$0	\$0	\$2,433,093.75	\$5,262,173.51	\$9,483,971.46	\$534,659	\$578,167	\$625,215	\$676,092	\$731,109	\$790,603
Total Revenue	\$0	\$20,275,781	\$46,284,540	\$84,295,269	\$30,141,247	\$22,872,920	\$24,734,204	\$26,746,950	\$28,923,483	\$31,277,131	\$33,822,308

16.5.2 Inventory Costs

Inventory Costs - in 2012 dollars

Base Case

Reagent Costs

CD45RA ab + dyna bead		\$7.73
	Cost per mL	\$10.30
	mL per sample	1
	Discount	25%
Lysis solution		\$0.13
PBS buffer		\$0.02
	Cost per mL	0.0211
	mL per sample	1.5
	Discount	25%
Calcium Chloride		\$0.01
	cost per mg	0.000265
	mg per sample	34
	Discount	25%
Thrombin		\$0.14
	cost per mg	382
	mg per sample	0.0005
	Discount	25%
TBS		\$0.05
	Cost per mL	0.0033
	mL per sample	20
	Discount	25%
Dry Ice		\$0.75
Total Costs		\$8.83

Microfluidic Chip

Total Microfluidic Chip Costs	\$5.00
-------------------------------	--------

Microfilter

\$1.00

Cartridge Costs

Plastic Box	1.5141
Total Cartridge Costs	1.5141

TOTAL COST PER CARTRIDGE \$15.34

Price of Liquid Handling Machine	\$75,000
% profit margin	85%
Cost of Liquid Handling Machine	\$11,250

Magnet 100

TOTAL COST PER PROCESSING UNIT \$11,350**Process Machinery**

Assembly Machine	100,000	
Freezer (-20 C)	6780	
Curing Oven	22400	
PDMS Sealing Machine	210,000	
Package/hermetically sealer	140,000	
Laboratory	180000	
	Cost per sq ft per month	10
	No months	12
	Sq ft of space	1500
Corporate	63000	
	Cost per sq ft per month	10
	No months	12
	Sq ft of space	525
Production	288000	
	Cost per sq ft per month	8
	No months	12
	Sq ft of space	3000
Total Rent Costs	531000	

Salary Costs

Salary/yr

Management	\$120,000
R&D	
Ph.D.	\$80,000
BSE	\$50,000
Finance	\$100,000
Engineer	\$60,000
Lawyer	\$120,000
Secretary	\$25,000
Total Salary Costs	\$655,000

R&D Costs

Cost

Clinical Trial costs	\$2,500,000
FDA Approval	\$500,000
R&D Start-up Costs	\$1,000,000
Lab Information Management Soft	\$100,000

TOTAL FIXED COSTS \$5,765,180

16.5.3 Operating Assumptions

Operating Assumptions

Base Case

	1 2013	2 2014	3 2015	4 2016	5 2017	6 2018	7 2019	8 2020	9 2021	10 2022	11 2023
Other Costs [f(revenue)]											
R&D	0.00%	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%
SG&A	5.00%	5.00%	4.00%	4.00%	4.00%	3.00%	3.00%	3.00%	3.00%	3.00%	3.00%
Space (Sq Ft)											
Lab Space	2000	2000	2000	2250	2250	2250	2250	2250	2250	2250	2250
Production Space	0	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000
Corporate Space	525	1500	1725	2025	2400	2500	2500	2600	2600	2600	2600
Process Machinery (# units)											
Assembly Machine	0	1	1	1	1	2	2	2	2	2	2
Freezer (-20 C)	1	1	1	1	1	1	1	1	1	1	1
Curing Oven	1	1	1	1	1	1	1	1	1	1	1
PDMS Sealing Machine	1	1	1	1	1	1	1	1	1	1	1
Change	1	0	0	0	0	0	0	0	0	0	0
Package/hermetically sealer	0	1	1	1	1	1	1	1	1	1	1
Change	0	1	0	0	0	0	0	0	0	0	0
Employees (# persons)											
Management	3	3	3	3	3	4	4	5	5	5	5
R&D	8	8	8	9	9	9	9	9	9	9	9
Ph.D.	4	4	4	4	4	4	4	4	4	4	4
BSE	4	4	4	5	5	5	5	5	5	5	5
Finance	1	2	2	3	3	3	3	3	3	3	3
Sales/Marketing	0	3	4	5	8	8	8	8	8	8	8
Production	0	8	10	12	14	14	14	14	14	14	14
Operator	0	3	4	5	6	6	6	6	6	6	6
Engineer	0	5	6	7	8	8	8	8	8	8	8
Lawyer	1	1	1	1	1	1	1	1	1	1	1
Secretary	1	2	2	2	2	2	2	2	2	2	2
Total	14	27	30	35	40	41	41	42	42	42	42
Chip Cost and Size											
Cost (2012 dollars)	\$5.00										

16.5.4 Inventory, Working Capital, PP&E

Inventory, Working Capital, PP&E
Base Case

	Inflation Rate 2.5%											MACRS Depreciation Schedule	
	1 2013	2 2014	3 2015	4 2016	5 2017	6 2018	7 2019	8 2020	9 2021	10 2022	11 2023		
Inventory													
Cartridge	\$ -	\$ 2,550,398	\$ 5,515,874	\$ 9,941,213	\$ 10,750,180	\$ 11,624,975	\$ 12,570,958	\$ 13,593,919	\$ 14,700,125	\$ 15,896,347	\$ 17,189,913		Y1 0.1
Processing Unit	\$ -	\$ 1,887,067	\$ 4,081,254	\$ 7,355,610	\$ 414,673	\$ 448,417	\$ 484,906	\$ 524,366	\$ 567,036	\$ 613,178	\$ 663,076		Y2 0.18
Total Inventory	\$ -	\$ 4,437,465	\$ 9,597,128	\$ 17,296,824	\$ 11,164,852	\$ 12,073,392	\$ 13,055,864	\$ 14,118,285	\$ 15,267,161	\$ 16,509,526	\$ 17,852,988		Y3 0.144
													Y4 0.1152
Working Capital													Y5 0.0922
Accounts Receivable	0.00	1,689,648.44	3,857,044.97	7,024,605.75	2,511,770.57	1,906,076.67	2,061,183.66	2,228,912.48	2,410,290.23	2,606,427.60	2,818,525.64		Y6 0.0737
Inventory	0.00	85,199.33	184,264.85	332,099.01	214,365.16	231,809.12	250,672.59	271,071.07	293,129.48	316,982.89	342,777.38		Y7 0.0655
Accounts Payable	0.00	387,966.85	829,998.19	1,474,700.36	963,024.34	1,035,748.90	1,114,910.16	1,203,239.41	1,298,681.56	1,401,146.25	1,512,044.79		Y8 0.0655
Cash reserves for salaries	95,427.08	160,621.35	178,328.76	210,635.48	239,748.55	256,672.53	262,422.67	280,500.60	286,846.45	293,350.95	300,018.05		Y9 0.0656
Net Working Capital	(95,427.08)	1,226,259.57	3,032,982.88	5,671,368.92	1,523,362.84	845,464.36	934,523.41	1,016,243.54	1,117,891.70	1,228,913.30	1,349,240.17		Y10 0.0655
Additional Working Capital	(95,427.08)	1,321,686.65	1,806,723.31	2,638,386.05	(4,148,006.08)	(677,898.48)	89,059.05	81,720.13	101,648.16	111,021.60	120,326.87		Y11 0.0328
													1
PP&E													
Beginning	\$ -	\$ 1,100,000	\$ 1,339,180	\$ 1,480,180	\$ 1,369,554	\$ 1,247,400	\$ 1,136,312	\$ 1,135,163	\$ 1,035,829	\$ 919,741	\$ 792,732		
Equipment Purchases	\$ 1,100,000	\$ 239,180	\$ 240,000	\$ -	\$ -	\$ -	\$ 100,000	\$ -	\$ -	\$ -	\$ -		
Maintenance CAPEX	\$ -	\$ 110,000	\$ 133,918	\$ 148,018	\$ 136,955	\$ 124,740	\$ 113,631	\$ 113,516	\$ 103,583	\$ 91,974	\$ 79,273		
Ending	\$ 1,100,000	\$ 1,449,180	\$ 1,713,098	\$ 1,628,198	\$ 1,506,509	\$ 1,372,140	\$ 1,349,943	\$ 1,248,679	\$ 1,139,412	\$ 1,011,715	\$ 872,005		
Less Accumulated Depreciation	\$ -	\$ 110,000	\$ 232,918	\$ 258,644	\$ 259,109	\$ 235,829	\$ 214,780	\$ 212,849	\$ 219,671	\$ 218,983	\$ 219,537		
Net PP&E	\$ 1,100,000	\$ 1,339,180	\$ 1,480,180	\$ 1,369,554	\$ 1,247,400	\$ 1,136,312	\$ 1,135,163	\$ 1,035,829	\$ 919,741	\$ 792,732	\$ 652,468		
Depreciation Schedule	Basis	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023		
2013E Depreciation Amount	\$ 1,100,000	\$110,000	\$198,000	\$158,400	\$126,720	\$101,420	\$81,070	\$72,050	\$72,050	\$72,160	\$72,050		
2014E Depreciation Amount	\$ 349,180		\$34,918	\$62,852	\$50,282	\$40,226	\$32,194	\$25,735	\$22,871	\$22,871	\$22,906		
2015E Depreciation Amount	\$ 373,918			\$37,392	\$67,305	\$53,844	\$43,075	\$34,475	\$27,558	\$24,492	\$24,492		
2016E Depreciation Amount	\$ 148,018				\$14,802	\$26,643	\$21,315	\$17,052	\$13,647	\$10,909	\$9,695		
2017E Depreciation Amount	\$ 136,955					\$13,696	\$24,652	\$19,722	\$15,777	\$12,627	\$10,094		
2018E Depreciation Amount	\$ 124,740						\$12,474	\$22,453	\$17,963	\$14,370	\$11,501		
2019E Depreciation Amount	\$ 213,631							\$21,363	\$38,454	\$30,763	\$24,610		
2020E Depreciation Amount	\$ 113,516								\$11,352	\$20,433	\$16,346		
2021E Depreciation Amount	\$ 103,583									\$10,358	\$18,645		
2022E Depreciation Amount	\$ 91,974										\$9,197		
2023E Depreciation Amount	\$ 79,273												
Total		110000	232918	258644.2	259108.96	235828.506	214780.3122	212849.3752	219671.3664	218983.2927	219536.6514		

16.5.5 Income Statement

Income Statement

Base Case

	Inflation Rate Tax Rate											
	2.50% 37.00%											
	1	2	3	4	5	6	7	8	9	10	11	
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	
Revenues	\$ -	\$ 20,275,781	\$ 46,284,540	\$ 84,295,269	\$ 30,141,247	\$ 22,872,920	\$ 24,734,204	\$ 26,746,950	\$ 28,923,483	\$ 31,277,131	\$ 33,822,308	
Cost of Goods Sold	\$ -	\$ 4,437,465	\$ 9,597,128	\$ 17,296,824	\$ 11,164,852	\$ 12,073,392	\$ 13,055,864	\$ 14,118,285	\$ 15,267,161	\$ 16,509,526	\$ 17,852,988	
Cartridge	\$ -	\$ 2,550,398	\$ 5,515,874	\$ 9,941,213	\$ 10,750,180	\$ 11,624,975	\$ 12,570,958	\$ 13,593,919	\$ 14,700,125	\$ 15,896,347	\$ 17,189,913	
Processing Unit	\$ -	\$ 1,887,067	\$ 4,081,254	\$ 7,355,610	\$ 414,673	\$ 448,417	\$ 484,906	\$ 524,366	\$ 567,036	\$ 613,178	\$ 663,076	
Gross Profit	\$ -	\$ 15,838,316	\$ 36,687,412	\$ 66,998,446	\$ 18,976,395	\$ 10,799,528	\$ 11,678,340	\$ 12,628,665	\$ 13,656,322	\$ 14,767,605	\$ 15,969,319	
% Margin												
Salaries	\$ 1,145,125	\$ 1,927,456	\$ 2,139,945	\$ 2,527,626	\$ 2,876,983	\$ 3,080,070	\$ 3,149,072	\$ 3,366,007	\$ 3,442,157	\$ 3,520,211	\$ 3,600,217	
Equipment Purchases	\$ 1,100,000	\$ 239,180	\$ 240,000	\$ -	\$ -	\$ -	\$ 100,000	\$ -	\$ -	\$ -	\$ -	
Depreciation	\$ -	\$ 110,000	\$ 232,918	\$ 258,644	\$ 259,109	\$ 235,829	\$ 214,780	\$ 212,849	\$ 219,671	\$ 218,983	\$ 219,537	
R&D Clinical Trial Costs	\$ 2,500,000	\$ 1,013,789	\$ 2,314,227	\$ 4,214,763	\$ 1,507,062	\$ 1,143,646	\$ 1,236,710	\$ 1,337,347	\$ 1,446,174	\$ 1,563,857	\$ 1,691,115	
Rent	\$ 303,000	\$ 708,000	\$ 735,000	\$ 801,000	\$ 846,000	\$ 858,000	\$ 858,000	\$ 870,000	\$ 870,000	\$ 870,000	\$ 870,000	
SG&A	\$ -	\$ 1,013,789	\$ 1,851,382	\$ 3,371,811	\$ 1,205,650	\$ 686,188	\$ 742,026	\$ 802,408	\$ 867,704	\$ 938,314	\$ 1,014,669	
EBIT	\$ (5,048,125)	\$ 10,826,102	\$ 29,173,940	\$ 55,824,601	\$ 12,281,591	\$ 4,795,796	\$ 5,377,751	\$ 6,040,052	\$ 6,810,615	\$ 7,656,240	\$ 8,573,781	
% Margin												
NOL begin	\$ -	\$ 5,048,125										
Adds/Subs	\$ 5,048,125	\$ (5,048,125)										
NOL End	\$ 5,048,125	\$ -										
Taxable Income	\$ (5,048,125)	\$ 5,777,977	\$ 29,173,940	\$ 55,824,601	\$ 12,281,591	\$ 4,795,796	\$ 5,377,751	\$ 6,040,052	\$ 6,810,615	\$ 7,656,240	\$ 8,573,781	
Taxes	\$ -	\$ 2,137,851.38	\$ 10,794,357.84	\$ 20,655,102.49	\$ 4,544,188.67	\$ 1,774,444.40	\$ 1,989,767.87	\$ 2,234,819.24	\$ 2,519,927.47	\$ 2,832,808.90	\$ 3,172,299.11	
Net Income	\$ (5,048,125)	\$ 8,688,250	\$ 18,379,582	\$ 35,169,499	\$ 7,737,402	\$ 3,021,351	\$ 3,387,983	\$ 3,805,233	\$ 4,290,687	\$ 4,823,431	\$ 5,401,482	
% Margin												

*16.5.6 Free Cash Flow*Free Cash Flow
Base Case

	1	2	3	4	5	6	7	8	9	10	11
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Net Income	(5,048,125.00)	8,688,250.32	18,379,582.26	35,169,498.83	7,737,402.33	3,021,351.27	3,387,983.13	3,805,232.77	4,290,687.31	4,823,431.36	5,401,482.27
Adjustments for non-cash items											
Depreciation	0.00	110,000.00	232,918.00	258,644.20	259,108.96	235,828.51	214,780.31	212,849.38	219,671.37	218,983.29	219,536.65
Additional Working Capital for year n	(95,427.08)	1,321,686.65	1,806,723.31	2,638,386.05	(4,148,006.08)	(677,898.48)	89,059.05	81,720.13	101,648.16	111,021.60	120,326.87
Operating Cash Flow	(5,143,552.08)	10,119,936.96	20,419,223.57	38,066,529.08	3,848,505.21	2,579,281.29	3,691,822.49	4,099,802.27	4,612,006.84	5,153,436.25	5,741,345.80
Less: Purchases of PP&E	1,100,000.00	349,180.00	373,918.00	148,018.00	136,955.38	124,740.02	213,631.17	113,516.26	103,582.95	91,974.11	79,273.19
Free Cash Flow	(6,243,552.08)	9,770,756.96	20,045,305.57	37,918,511.08	3,711,549.83	2,454,541.27	3,478,191.32	3,986,286.01	4,508,423.89	5,061,462.15	5,662,072.61

16.5.7 Valuation and Returns

Valuation and Returns
Base Case

	1	2	3	4	5	6	7	8	9	10	11
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Free Cash Flow	-6,243,552	9,770,757	20,045,306	37,918,511	3,711,550	2,454,541	3,478,191	3,986,286	4,508,424	5,061,462	5,662,073
Terminal Value											34,886,461
Total	-6,243,552	9,770,757	20,045,306	37,918,511	3,711,550	2,454,541	3,478,191	3,986,286	4,508,424	5,061,462	40,548,534
PV (2012 dollars)	-5,371,722	7,232,555	12,766,092	20,776,784	1,749,702	995,545	1,213,740	1,196,803	1,164,557	1,124,847	7,753,085
NPV (2012 dollars)	50,601,988										
Value of Company	58,698,306.25	68,360,315.14	79,455,194.29	92,350,772.32	107,339,302.67	124,760,471.49	145,009,096.02	168,544,072.30	195,898,775.24	227,693,146.46	264,647,744.13
Series A	-\$6,243,552	\$0	\$0	\$0	\$21,467,861	\$0	\$0	\$0	\$0	\$113,846,573	
IRR	45%										

Perp. Growth rate	0.00%
Risk Free Rate (10 year treasury bill)	2.23%
Market Risk Premium	7.00%
Beta	2
Discount Rate	16.23%
NPV	\$50,601,988
IRR to Series A Investor	45%

16.5.8 Payback Period

Payback Period
Base Case

	Simple I = Discounted I =	0.0% 16.2%	1st Year of Production										
			1	2	3	4	5	6	7	8	9	10	11
			2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Free Cash Flow			(6,243,552)	9,770,757	20,045,306	37,918,511	3,711,550	2,454,541	3,478,191	3,986,286	4,508,424	5,061,462	5,662,073
Cumulative PW at i=0%/year			(6,243,552)	3,527,205	23,572,510	61,491,022	65,202,571	67,657,113	71,135,304	75,121,590	79,630,014	84,691,476	90,353,549
Simple Payback Period				Payback year									
PW of cash flow at i=discount rate			(5,371,722)	2,610,924	15,012,434	33,692,928	30,737,853	27,441,259	24,823,186	22,553,756	20,568,975	18,821,634	17,276,056
Cumulative PW at i=discount rate			(5,371,722)	(2,760,798)	12,251,637	45,944,565	76,682,418	104,123,677	128,946,864	151,500,619	172,069,594	190,891,228	208,167,284
Discounted Payback Period				Payback year									

