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High Throughput Screening of Clopidogrel Resistance Using Microfluidic Technology

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Abstract
The pre-treatment of patients with clopidogrel before primary percutaneous coronary intervention (PCI) has been shown to lower the risk of complications that could lead to heart attack or stroke during the procedure. However, the proper administration of clopidogrel requires the measurement of the patient's drug resistance due to its inherent variation across the population. Approximately 1.1 million PCIs were performed in the US alone in 2008. As the patient population is becoming increasingly aware of the benefits of clopidogrel treatment prior to PCI, there is an ever-expanding market potential for clopidogrel resistance screening devices. As most of the existing devices utilize traditional test-tube-scale bench-top technology that usually sets limitations on the throughput and applicability of the test itself, the market demands a device that not only minimizes the cost per test but also produces consistent and comprehensive results. In this report, guided by the innovation map, we are able to link soft lithography in combination with micro-patterning technology to the customer's requirements, and come up with a higher-throughput system that meets the market demand. Our system consists of two parts: the chip and the device. We focus our design effort primarily on the chip, in which micro-channel layout, dry reagent dissolution, reagent mixing and reservoir volume design are carefully worked out. On the other hand, the design of the device is discussed briefly, but production is assumed to be outsourced. With the cost estimates from suppliers and the assumed expected market share to be 50%, the net present value is computed to be about 45 million, indicating a lucrative return to investors.

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April 14, 2009

Professor Leonard Fabiano
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Dear Professor Fabiano and Dr. Diamond,

Our group was presented with the task of designing a point-of-care system that uses an electronic device and disposable microfluidic chips to determine a patient's resistance to the anti clotting drug Plavix using a small blood sample. We succeeded in designing such a system that has a high throughput and a low cost, and it has been termed the "Multiple Channel Coagulation Resistance Assay System", or MCCRA System. In comparison to existing products with similar functions, our device proves to be less expensive, easier to use, and it provides more comprehensive results.

The MCCRA System gives an output reading for four different concentrations of the anti-clotting reagent, MRS 2395. The total market capture is expected to be around 50% of the 1.1 million patients in the U.S. undergoing Percutaneous Coronary Interventions (PCIs) each year. With the price of $100 per chip, our annual sales achieve a remarkable amount of $50 million, net the annual costs of operation and the initial capital investment to give a Net Present Value of about 45 million, suggesting a profitable project.

Yours sincerely,

Amanda Abbott

Elizabeth Kohli

Zhenteng Li

Paul O'Brien

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CBE 459 Advisor: Professor Leonard A. Fabiano
Project Advisor: Dr. Scott Diamond, assisted by Sean Maloney
Project Suggested By: Dr. Scott Diamond

Department of Chemical and Biomolecular Engineering, University of Pennsylvania, 2009

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Part 1 **Introduction**

1.1 Abstract

The pre-treatment of patients with clopidogrel before primary percutaneous coronary intervention (PCI) has been shown to lower the risk of complications that could lead to heart attack or stroke during the procedure. However, the proper administration of clopidogrel requires the measurement of the patient's drug resistance due to its inherent variation across the population. Approximately 1.1 million PCIs were performed in the US alone in 2008. As the patient population is becoming increasingly aware of the benefits of clopidogrel treatment prior to PCI, there is an ever-expanding market potential for clopidogrel resistance screening devices. As most of the existing devices utilize traditional test-tube-scale bench-top technology that usually sets limitations on the throughput and applicability of the test itself, the market demands a device that not only minimizes the cost per test but also produces consistent and comprehensive results. In this report, guided by the innovation map, we are able to link soft lithography in combination with micro-patterning technology to the customer's requirements, and come up with a higher-throughput system that meets the market demand. Our system consists of two parts: the chip and the device. We focus our design effort primarily on the chip, in which micro-channel layout, dry reagent dissolution, reagent mixing and reservoir volume design are carefully worked out. On the other hand, the design of the device is discussed briefly, but production is assumed to be outsourced. With the cost estimates from suppliers and the assumed expected market share to be 50%, the net present value is computed to be about 45 million, indicating a lucrative return to investors.

1.2 Introduction

1.2.1 Motivation

In 2008, 1.1 million Americans with circulatory complications underwent Percutaneous Coronary Intervention (PCI) procedures. Often, the success rate of PCI is limited by the risk of adverse clotting events. Research supported by angiographic evaluation evidence has shown that clopidogrel (Plavix)[1] can reduce these events during the procedure and chances of developing further complication if administered prior to the procedure. However, some patients exhibit poor response to the drug and require a higher dose to achieve a therapeutic level of the active drug metabolite in circulation. The current evaluation of Plavix resistance requires laboratory scale manual mixing of the drug with the patient's blood and the subsequent observation of platelet function. Progress has been made to increase the throughput and the cost-effectiveness of this test, yet none of the existing technologies satisfies the customer's desires of small blood volume, high throughput and automated operation.

Since its discovery and development by Bristol-Myers Squibb, clopidogrel has been marketed as Plavix worldwide to 110 different countries with annual sales of $5.9 billion and exhibiting growth rate of 20%. (1) The market potential for Plavix resistance screening devices is becoming apparent. The goal of this design project is to come up with an automated machine that uses disposable microfluidic chips to quantify the patient's resistance to Plavix based on a blood volume of less than 1 milliliter.

[1] "clopidogrel" is the generic name for Plavix and both terms will be used interchangeably throughout this report.

1.2.2 Project Charter and Scope

Project Name	Multiple Channel Coagulation Resistance Assay System (MCCRA System)
Project Champions	Scott Diamond, PhD; Sean Maloney
Project Leaders	Amanda Abbott, Elizabeth Kohli, Zhenteng Li, Paul O'Brien
Specific Goals	Develop a Point of Care Device that measures a patient's resistance to MRS 2395, which can be translated into clopidogrel dosing by a physician
Project Scope	<u>In-scope:</u> -Disposable microfluidic chip that analyzes amount of blood clotting for 4 different MRS 2395 concentrations -Manufacturing procedure and daily operational schedule for microfluidic chip production -Basic design of a small device that will analyze amount clotting on chip using light transmittance -Economic analysis/plan for production/distribution of system -Test disposable chip design in laboratory setting <u>Out-of-Scope:</u> -Adapting device to be used with reagents other than MRS 2395 -Detailed electronic development of interior of device
Deliverables	Business opportunity Market expansion Technical feasibility Manufacturing capability assessment Competitive product analysis Laboratory data analysis
Timeline	-The project feasibility and design stages took place over the course of approximately 3 months (contents of this report). -Starting in 2010 and lasting for one year is a design period in which the structure and components of the device will be tested and improved. -Following the year of design, there will be two years during which limited production will occur while clinical trials are conducted and FDA approval is obtained -In the first year following FDA approval there will be limited distribution of the system to major hospitals for further testing and the beginning marketing stages -After the year of distribution the manufacturing will be at 100% capacity and the device will be on sale nationally for 6 years. -After the six years of sales the profitability will be analyzed and the future of the project will be determined and possibility of expansion considered.

1.2.3 Technology-Readiness Assessment

An innovation map (See Figure 1-1) 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. [(2)]

The innovation map shows the manufacturing of the Multi-Channel Coagulation Resistance Assay (MCCRA) System which consists of a microfluidic chip and a device to run the physical test and display the results to the practitioner. The technologies used to fabricate these items include soft lithography and replicate molding for the chip and outsourcing for the device. The technical differentials are the features that set the MCCRA System apart from other competing products. The ability to test for varying resistances in patients on a single chip is a new concept that makes this new product very valuable. The device feature for a quick turnaround test time is important for hospitals as well as patients. All of these specifics will be discussed in more detail later on. The market and innovative features of the system make these new technologies justifiable.

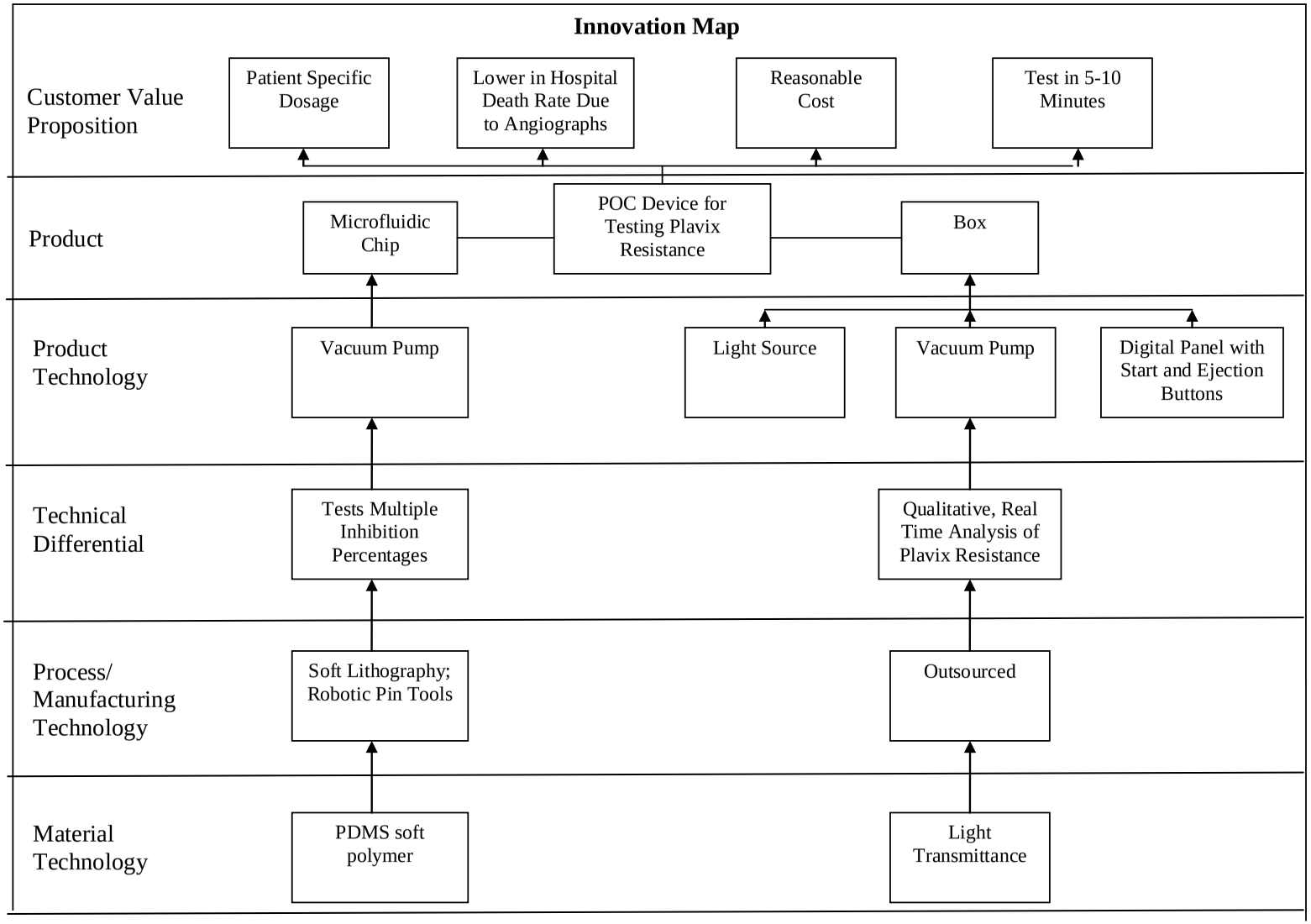


Figure 1-1 Innovation Map

The innovation map shows the new technologies that will be used in a product.

1.2.4 Report Contents Summary

Our design report can be broken down into four major parts. Part 1 addresses the motivation behind this project, presents a project charter, and briefs on the innovation map that guides us through the development of our product. Part 2 reviews the physiology of blood clots, the molecular pathways of clopidogrel, chip manufacture, and market potential analysis. Part 3 deals in depth with chip design, including chip design and manufacture, financial analysis, and a summary of the box components in succinct terms since the focus of this project is on the chip. Part 4 describes the post-design considerations, which include FDA approval and areas of future research and development.

Part 2 **Concept Stage**

2.1 Physiology of Blood Clotting

2.1.1 The Basics of Blood

The blood flowing through the human body, known as whole blood, has four main components: plasma, red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes). The plasma comprises about 55% of the whole blood, while the other 45% consists of the three blood cells.

Plasma is about 90% water, with the other 10% consisting of substances such as proteins, electrolytes, hormones, vitamins and cholesterol. This liquid is responsible for transport of blood components through the body.

Red blood cells are the second most prevalent component of whole blood, accounting for about 40-45% of its make-up. These cells are primarily responsible for the transport of carbon dioxide and oxygen to and from the lungs, respectively, through the protein hemoglobin found inside the cells.

White bloods cells compose only about 1% of the total volume of whole blood, and are responsible for protecting the body against infection.

Platelets are anuclear blood cells that gather at the location of an injury to allow coagulation to occur at the site. Deviations in the platelet count from normal values (300,000 to 600,000 per μL) can be very dangerous to the body. Too many platelets can lead to unwanted clotting, which can in turn cause heart attack or stroke. Too few platelets, however, can lead to the inability to clot normally and thus cause excessive bleeding. [(3)]

2.1.2 Hemostasis

Hemostasis refers to the complex network of steps leading to the formation of a clot at the site of a damaged blood vessel. Its purpose is to stop and prevent further loss of blood at the damaged location. [4] The process involves three major components which are platelets, endothelial cells, and blood clotting proteins. Under normal conditions (i.e. no damage to the blood vessel), endothelial cells line the interior of the blood vessel and prevent clots from forming on the walls by acting as a physical barrier to the formation as well as by releasing nitric oxide, prostacyclin and other molecules that inhibit platelet aggregation, while platelets and blood-clotting proteins are found in the blood plasma in an "inactive form" until activated by injury. [5] [6] Hemostasis can be broken down into three categories, known as primary hemostasis, secondary hemostasis and tertiary hemostasis. It is important to note that all three categories in fact occur simultaneously. They are only divided in this way for easier understanding of mechanisms, not to delineate three sequential steps in the process. [7]

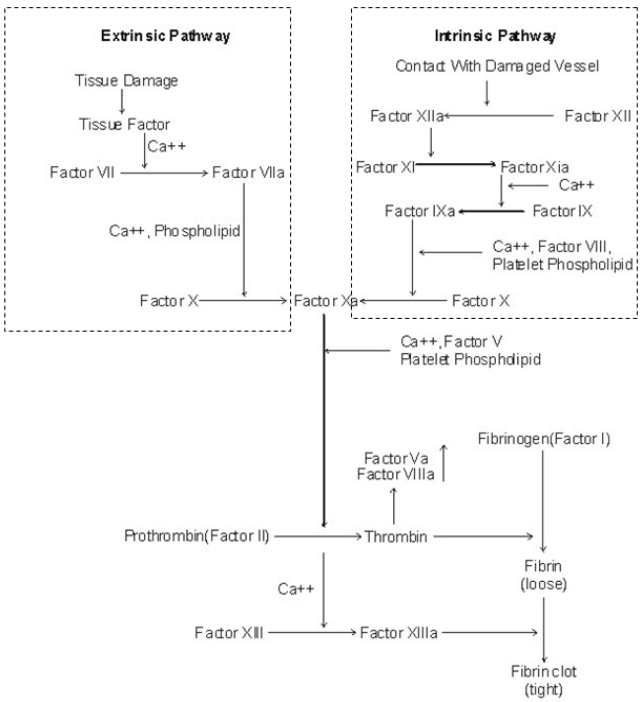


Figure 2-1 Coagulation Cascade
The solid black lines represent activating factors and the complexes that they activate.

Primary hemostasis refers to the formation of the platelet plug. When injury occurs, the layer of endothelial cells within the vessel is broken and subendothelial surfaces are exposed. Without the inhibition of the endothelial cells, platelets begin to adhere to the wall

of the vessel at the damaged location. The rate of platelet adhesion at this site is increased by the local vasoconstriction that is triggered upon breakage of the blood vessel. [6] Vasoconstriction slows down blood flow and reduces the rate of blood lost by narrowing the blood vessel in the injured area. Simultaneously, another area of the vessel is subjected to vasodilation, or expansion, so as to balance the narrowing effect. [8]

In the high shear rate conditions that exist at the site of an injury, von Willebrand factor (vWf) is responsible for platelet adhesion to the subendothelium by binding with glycoprotein Ib-IX which is found in the membrane of the platelets. (In areas such as the veins where low sheer rate conditions exist, the same process is controlled by collagen exposed during injury, not vWf. Instead, the fibrinogen binds to a platelet receptor known as glycoprotein Ia/IIa, or integrin $\alpha_2\beta_1$). Upon adhesion to the wall of the blood vessel, the platelets are activated, causing them to morph. The change in shape triggers the activation of glycoprotein IIb/IIIa (or integrin $\alpha_{IIb}\beta_3$a fibrinogen receptor located on the platelet's surface). In addition to shape change, the activated platelets release dense granules (which contain serotonin and ADP), thromboxane A_2 (TXA_2) and plated activating factor (PAF). The latter two secretions are vasoconstriction triggers as well as agonists for platelet aggregation. The platelet agonists TXA_2, PAF, ADP, and serotonin activate additional platelets, causing them to attach to those that have already adhered to the vessel wall. Thrombin, which is created via the coagulation cascade (which will be explained later), also acts as a platelet agonist. Fibrinogen serves as the primary mediator of platelet aggregation by binding to $\alpha_{IIb}\beta_3$on two adjacent platelets, thus forming the primary platelet plug. [6]

Secondary hemostasis refers to the production of fibrin via the coagulation cascade (See Figure 2-1).[9] The cascade is commonly broken down into the extrinsic pathway, the intrinsic pathway, and the common pathway. (10)

The extrinsic pathway is responsible for the initiation of the coagulation cascade *in vivo*. First, tissue factor is released from monocytes, endothelial cells, and subendothelial cells (the amount released is amplified by cytokines). Then, tissue factor binds to factor VII, which in turn activates factor X to Xa. Factor Xa, small levels of constitutively active factor Va, platelet phospholipids, and calcium activate the conversion of prothrombin to thrombin. Although tissue factor pathway inhibitor quickly inhibits this conversion, only the small amount of thrombin that is created is needed in order to activate the common pathway.[10] The intrinsic pathway is not believed to have a function *in vivo*, but is responsible for initiation from negatively charged synthetic surfaces, such as glass or plastic.

While the purpose of the extrinsic pathway is to initiate the coagulation cascade, the common pathway is responsible for amplifying the cascade. This amplification is triggered by the thrombin that is produced by the extrinsic pathway. The thrombin activates factor IX and factor VIII to XIa and VIIIa. Then, VIIa and IXa, phospholipids, and calcium all act to amplify the activation of factor X. By amplifying factor Xa and Va production, large amounts of the prothrombinase complex (XaVa) are formed and large amounts of thrombin are generated. The thrombin then cleaves fibrinogen into soluble fibrin monomers, which polymerize into fibrin protofibrils which then will polymerize into fibrin fibers which add mechanical stability to the forming platelet plug. Factor XIII, which is also activated by thrombin, then works with calcium to stabilize the fibrin polymer by cross-linking the fibrin fibers. The stabilized polymer is insoluble cross-linked fibrin.[10]

Tertiary hemostasis refers to the formation of the enzyme plasmin, which is responsible for breaking down the blood clot (known as fibrinolysis). When the coagulation cascade is activated, endothelial cells release tissue plasminogen activator, or tPA. The tPA converts the plasminogen found in the clot into plasmin. The plasmin then creates fibrin and fibrinogen degradation products by proteolytically cleaving the fibrin and fibrinogen that are in the clot. However, in order for tPA to activate the plasminogen, fibrin must be present. This restricts activation to only where thrombus is formed, keeping the tPA from undesirably destroying fibrinogen, factor V or factor VIII. In addition to tPA, plasminogen can be activated by urokinase, kallikrein or factor XIIa. [11]

2.2 Clopidogrel (Plavix)

2.2.1 Mechanism of Action

Adenosine diphosphate (ADP) binds to platelet G-protein linked surface receptors $P2Y_1$ and $P2Y_{12}$ (Figure 2-2). Upon binding, the G_q linked $P2Y_1$ receptor activates phospholipase C and causes the cytosolic surge of Ca^{++}, which is responsible for the immediate conformational change. The activated G_i linked receptor $P2Y_{12}$ detaches its α subunit from the β subunit, which lead to two independent signaling pathways. The α subunit inhibits adenylyl cyclase (AC), which catalyzes the formation of cyclic adenosine monophosphate (cAMP). The reduced level of cAMP causes a decreased

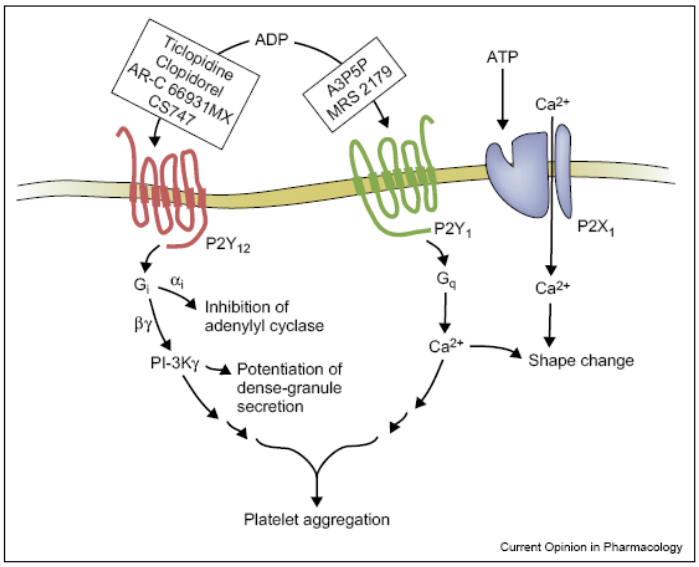


Figure 2-2: ADP mediated activation of platelet aggregation (14)

level of the specific protein kinases that phosphorylate the vasodilator-stimulated phosphoprotein (VASP). The phosphorylated form of VASP inhibits GP IIb/IIIa receptor, which is responsible for platelet aggregation. In contrast, the β subunit activates the phosphatidylinositol 3-kinase (PI3K) which is an important intermediate signaling molecule for dense and α-granule secretion. All told, P_2Y_{12} signaling both induces inside-out signaling, activating the $\alpha_{IIb}\beta_3$ integrins, and induces granule release.

Clopidogrel requires oxidation by the hepatic cytochrome *P450* to result in the opening of the thiophene ring and the forming of a carboxyl and thiol group. However, only a small proportion of the clopidogrel undergoes oxidation by *CYP450* in vivo. [12] The thiol group irreversibly binds to the platelet G_i-protein linked ADP surface receptor, $P2Y_{12}$, through a covalent disulfide bridge, thus reducing the number of inducible $P2Y_{12}$ receptors on the platelet surface that indirectly disable $\alpha_{IIb}\beta_3$ activation. Without the activated $\alpha_{IIb}\beta_3$ integrins, fibrin and fibrinogen cannot bind platelets together.

2.2.2 Variability of Clopidogel Resistance

Five to ten percent of the patients treated with clopidogrel exhibit resistance. Of that five to ten percent, 25% of these patients are reported to be partially responsive in standard platelet assays. [13] The causes of clopidogrel resistance cannot yet be elucidated and are still areas of active research. Yet many studies seem to agree that there are certain areas of research that seem most relevant to clopidogrel resistance: 1) genetic variation of the $P2Y_{12}$ and P-450 CYP3A; 2) extent of $P2Y_1$-dependent platelet aggregation; 3) accelerated platelet turnover; 4) interaction with medication that involves cytochrome *P-450 CYP3A4*.

2.2.2.1 Polymorphisms of $P2Y_{12}$ and P-450 CYP3A

There are 2 haplotypes, designated H1 and H2, associated with $P2Y_{12}$ that have been identified. The H2 haplotype is associated with increased maximal platelet aggregation in response to ADP. [14] Variations of the promoter region of $P2Y_{12}$ have been observed to increase transcriptional efficiency in the H2 haplotype; this partially explains why H2 carriers exhibit higher number of $P2Y_{12}$ receptors on the platelet surface than non-carriers.

Likewise, the variation of *CYP3A* expression in different individuals has been identified. The genetic basis for this difference has not yet been identified, but it is possible to use the erythromycin breath test to determine an individual's *CYP3A* activity. [15] Lau et al. has shown that the positive correlation between *CYP3A* activity and clopidogrel's effectiveness.

2.2.2.2 Extent of $P2Y_1$-Dependent Platelet Aggregation

Studies have found that activation via the $P2Y_1$ receptor alone can induce transient platelet aggregation. [14] The expression level of $P2Y_1$ receptor on the platelet surface is different across the patient population.

2.2.2.3 Different Platelet Turnover Rates

In times of stress, the bone marrow has accelerated platelet production. The replenished platelets carry $P2Y_{12}$ receptors that are unexposed to clopidogrel. [13] This dramatically decreases the duration of clopidogrel's anti-clotting effectiveness.

2.2.2.4 Interaction with Other Medications Involving P450

Drugs that involve cytochrome P450 have been postulated to reduce the effectiveness of clopidogrel. For example, studies have shown that atorvastatin (Lipitor), used to treat hypercholesteremia, also requires *P-450 CYP3A4* metabolism to become active. [16] Patients with

atherosclerotic disease are often treated for hypercholesteremia with both atorvastatin and clopidogrel. The former is shown to competitively inhibit the latter's anti-platelet activities.

2.2.3 Time Dependent Plavix Resistance

The percentage of platelet aggregation at a certain time post clopidogrel treatment varies across patient population and resistance to the drug diminishes given more time. For example, Gurbel et al. correlates the frequency of patients and absolute change in platelet aggregation after the drug treatment at 2 hr, 24 hr, 5 days and 30 days; the percentage of patients who are resistant to clopidogrel is found to be 63%, 31%, 31% and 15% respectively, where resistance is defined empirically by the study.[17] One possible explanation is that each patient's hepatic cytochrome *P450 CYP3A* rate of metabolism is different.

2.3 Chip Manufacture with PDMS

The microfluidic chip that will be at the heart of the POC device will be constructed of an elastomer known as polydimethylsiloxane. Abbreviated as PDMS, it possesses an array of qualities that make it an ideal choice for use in fabrication. First, features with sub 0.1 micron fidelity can be formed, meaning that many channels of small width are able to be fabricated on a single chip. Second, it is thermally and electrically insulating. Third, polydimethylsiloxane is mostly non-reactive towards reactants used. Also, PDMS possesses good bio-compatibility and can easily be surface-modified to make it biologically inert, which is an especially important characteristic in our system. In addition, the Young's Modulus is tunable, allowing one to adjust the elastomeric properties of PDMS to that required for the system. Finally, and most important, a layer of PDMS is optically transparent for a wide range of light wavelengths, down to 240 nanometers. The usefulness of this last characteristic of PDMS is useful because a detection system involving visible light will be used for monitoring the blood clotting.

Polydimethlysiloxane will be purchased from any number of chemical suppliers, and, once large-scale manufacturing commences, will be purchased in massive bulk installments. PDMS is supplied in a two parts: a base and curing agent. When the two are mixed, the silicon hydride groups present in the curing agent react with vinyl groups present in the base, polymerizing and forming an elastomeric solid. The base to curing agent proportions are adjustable values, which lead to different degrees of elastomeric solids and thus forms the basis for the tunable Young's Modulus of PDMS.

The manufacture of PDMS begins by the creation of a mask, or master. This mask has a reverse image of the intended pattern and is usually created via photolithography. Once the PDMS is mixed using the desired amounts of base and curing agents, it is poured onto the mask and allowed to harden. The device is peeled off the mask and attached to another substrate for further patterning.

An important manufacturing advantage of PDMS is its ability to be sealed to other homogenous or heterogeneous layers of PDMS, which might be necessary in the construction of a multilayer device. In addition this sealing can be done either reversibly or irreversibly, making the possibility of peeling the layers apart to inspect the device after use possible.

By using PDMS, the ability to mold a number of components becomes possible. Upon fabrication, valves, pumps, mixers, switches and a number of other miniaturized components can be fabricated. Moving up a level of complexity, the potential exists for the combination of these in sequence to form microfluidic unit operations. [18]

2.4 Lap-On-a-Chip Microfluidic Platform

Lab-on-a-chip technology involving microfluidics is continuously growing in popularity, especially in the biotechnological, medical, and pharmaceutical industries. By definition, microfluidics deals with the behavior, flow, and precise control of fluids that move through channels and areas that are of a micro-, nano-, or pico- scale. Features of a microfluidic setup include small volumes and sizes as well as low energy consumption.

Microfluidic platforms have many appealing features, such as a simple and well defined set of fluidic unit operations and a low cost for fabrication. Some examples of fluidic unit operations are fluid transport, fluid mixing, and separation or concentration of molecules. "Ideally, the set of unit operations will be connected in a monolithically integrated way so the platform allows for a seamless and simple integration of different fluids, which will give the platform a significant advantage over more complicated models" [(19)].

2.5 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). [(2)] Table 2-1 shows the desired customer requirements.

Customer Requirement	Product Requirements	Type	Weighting Factor (%)
High Throughput	Multiple tests simultaneously Small Sample Fast turnaround time	NUD	40
Accuracy	Instrument/measurement quality Reagent quality Sample quality	FTS	35
Sensitivity	Detection limit	FTS	15
Low Cost	Small reaction amounts	NUD	10

Table 2-1 Customer Requirements
The table above lists the customer requirements compiled from market research. Each requirement corresponds to product requirements, a type, and a weighting factor.

2.6 Critical-to-Quality Variables—Product Requirements

2.6.1 Critical-to-Quality (CTQ) 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 such as Accumetrics VerifyNow $P2Y_{12}$ Assay, Siemens PFA-100 System, and Helena AggRAM Module and by reviewing market survey data. The technical requirements and target values are shown below in Table 2-2.

Customer/Product Requirement	Technical Requirement (CTQ)	Target (per chip)
Multiple Tests Simultaneously	Multiple Concentrations of MRS 2395	4 concentrations of MRS 2395
Small Sample	Whole Blood Samples	360 µL
Fast Turnaround Time	Total Assay Time	~ 5 minutes + 10 minute incubation
Instrument/Measurement Quality	Advanced Error Verification System	Multiple Tests/[MRS] 2 channels/MRS concentration
Reagent Quality	Chemical Purity	> 95% pure
Sample Quality	Directly From Patient	Anti-coagulated blood
Detection Limit	Visible Light Detection	380-750 nm
Small Reaction Amounts	ADP Volume MRS 2395 Mass Collagen Volume	160 nL 0.06327 mg 32 nL

Table 2-2 Critical-to-Quality Variables
The product requirements are translated into technical requirements and given a target to reach.

2.6.2 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 designate that both variables are increasing or decreasing while a minus sign is used to show if one variable increases while the other one decreases or vice versa. If no relationship exists between the variables, the space is left blank. For the House of Quality in Figure 2-3, the technical requirements do not directly relate to each other so the top of the house is blank. The final section displays the weighting factors for the customer requirements which were already determined in the customer requirements table. [(2)]

										1	Collagen Volume
									1		MRS 2395 Volume
								1			ADP Volume
							1				Visible Light
						1					Directly From Patient
					1						Chemical Purity
				1							Advanced Error Verification System
			1								Total Assay Time
		1									Whole Blood Samples
	1										Multiple Concentrations of MRS 2395
Customer Requirement	Multiple Concentrations of MRS 2395	Whole Blood Samples	Total Assay Time	Advanced Error Verification System	Chemical Purity	Directly From Patient	Visible Light	ADP Volume	MRS 2395 Volume	Collagen Volume	Weighting Factor
High Throughput (NUD)											0.40
Multiple Tests Simultaneously	x										
Small Sample		x									
Fast Turnaround Time			x								
Accuracy (FTS)											0.35
Instrument/Measurement Quality				x							
Reagent Quality					x						
Sample Quality						x					
Sensitivity (FTS)											0.15
Detection Limit							x				
Low Cost (NUD)											0.10
Small Reaction Volume								x	x	x	

Figure 2-3 House of Quality

The House of Quality relates the customer requirements to the overall product requirements. The customer requirements and weighting factors are highlighted in green and the technical requirements are highlighted in light orange.

2.7 Product Concepts

"The Pugh Matrix is used to compare how well the NUD requirements are satisfied by the device concept versus its leading competitor" [(2)]. Based on data accumulated from researching the market, it was determined that Helena's AggRAM Module and Analyzer is the main rival of the MCCRA System. The requirements are listed and the current solutions to those requirements used by the AggRAM Module are presented in Table 2-3. Our system's alternative for each requirement is ranked as inferior, superior, or equal to our competitor's solution. As shown in Table 2-3, the MCCRA System has many superior concepts when compared to the competition such as a better error detection system, faster turnaround time, lower cost, and smaller sample.

Requirement	Reference Concept (Helena's AggRAM Module and Analyzer)	MCCRA System
Multiple Tests Simultaneously	Up to 4 tests simultaneously	0
Small Sample	900 µL	+
Fast Turnaround Time	5 minutes plus 45 minute preparation/incubation time	+
Advanced Error Detection System	Baseline channel for 100% aggregation	+
Low Cost	$7,995 for device and $14,995 for analyzer $70 for reagents and cuvettes	+

Table 2-3 Pugh Matrix
The Pugh Matrix compares our product against its competitor in regards to fulfilling requirements.

2.8 Market and Competitive Analysis

2.8.1 Accumetrics—VerifyNow $P2Y_{12}$ Assay

The Accumetrics VerifyNow System uses an optical detection system and a single channel assay to measure platelet aggregation. The $P2Y_{12}$ Assay, one form of a test that can be performed by the system, is designed to measure how well clopidogrel blocks the $P2Y_{12}$ receptors on the platelets by using light transmittance. This test is performed using the whole blood of a patient who is already being medicated with clopidogrel.

The $P2Y_{12}$ Assay uses 2 mL of whole blood and has a 3 minute runtime, not including an incubation period of about 10 minutes. During the test, the adenosine diphosphate (ADP) is used as an agonist to induce the activation of platelets which are present in whole blood. The activated platelets attach to the fibrinogen coated beads and aggregation occurs. While this reaction is occurring in one channel, a baseline channel containing thrombin and fibrinogen coated beads is being run simultaneously. Thrombin receptors are strong platelet activators that work independently from the $P2Y_{12}$ receptor so this baseline channel is used as the control.

Light transmittance is the method of detection used for this system. The change in optical signal in the assay channel is measured and reported in $P2Y_{12}$ Reaction Units (PRU). The change in transmittance is also measured in the baseline channel. The percent inhibition is calculated by the percentage difference between the PRU and baseline channel results.

Figure 2-4 Accumetrics VerifyNow System [20]

The list price for the VerifyNow System is $8,000 and the Assay Kit costs $1,250 for 25 tests. The system is also beneficial because it can be used with assay kits involving aspirin as well as $\alpha_{IIb}\beta_3$ inhibitors. [20]

2.8.2 Siemens—PFA-100 System

The Siemens PFA-100 System detects platelet dysfunction and is the first commercially available *in vitro* test to incorporate the high-shear flow as well as platelet adhesion and aggregation that would occur after a vascular injury. This test is designed to decipher the amount of time in which the platelets will coagulate and close a hole which represents a vascular injury. It is assumed the patient is already taking clopidogrel in order for this test to give usable results.

This test uses 800 µL of citrated whole blood and has a runtime of 5 minutes, not including a 15 minute incubation period. The system uses a membrane of collagen and ADP to monitor platelet interaction. The whole blood flows through a 150 µm port through the collagen/ADP membrane at a shear rate of 4,000-5,000/s. The time required for the platelets to close the aperture shows the platelet hemostatic capacity. [21]

Figure 2-5 Siemens PFA-100 System (21)

The list price for the PFA-100 System is $15,000 and the ADP/Collagen Test Cartridges as well as the ADP/Epinephrine Test Cartridges are sold in pack of 20 that cost $300 each.

2.8.3 Helena—AggRAM Module and Analyzer

The Helena AggRAM device uses light transmittance to analyze platelet aggregation in four channels at once and specialized software to graph the results. The four channels can run tests for various dilutions of one patient's blood or run 4 separate patient samples at the same time. The device links to the software and automatically calculates the slope, max % aggregation, and time to reach max % aggregation. The lag phase is displayed on the computer along with graphs and curves which can be printed out or saved for documentation. A database exists for the storage of results and graphs for quality control purposes. This device assumes that the patient is already taking clopidogrel but could potentially be modified by adding different concentrations of the anticoagulant to the various cuvette samples. [22]

The run time for the AggRAM Module is about 3 minutes with a 45 minute incubation time. 450 µL of whole blood is needed for each cuvette, but generally at least 900 µL is used because one of the cuvettes will be used as a baseline with 100% coagulation. The procedure begins forming the test sample by mixing the whole blood and the anticoagulant, sodium citrate. This mixture is centrifuged for 10-15 minutes and then sits for 30 minutes before the test can be performed. The baseline sample does not contain anticoagulant but was also centrifuged for 10-15 minutes. During this time period a platelet count is performed so the platelets in each sample can be standardized, usually to 250,000/mm^3. To begin the test, ADP, collagen, and epinephrine are added to all samples except the baseline sample along with a stir bar and then incubated for 1-3 minutes. The cuvettes are placed in the module and the light transmittance begins. [23]

A laser diode is used to conduct light transmittance detection. The change in absorbance along with the increased light transmittance is measured as platelet aggregation occurs. The percent of aggregation is calculated using the following equation:

$$\%\ \boldsymbol{aggregation} = \frac{\boldsymbol{O.D._{initial} - O.D._{maximum}}}{\boldsymbol{O.D._{initial}}} * \mathbf{100} \qquad \textbf{(2-1)}$$

where O.D. is the optical density. The sample's results can then be compared to the baseline sample and stored graphs of patients that had normal platelet aggregation.

The price of the device is listed as $7,995 and the analyzer software costs $14,995. The reagent kit containing ADP, collagen, and epinephrine costs $116 and accounts for 2 full tests. An additional cost must be factored in for buying the silicon coated cuvettes and a one-time cost for the magnetic stir bars.

Figure 2-6 Helena Laboratories AggRAM Module and Analyzer (22)

2.8.4 Conclusions

After comparing the MCCRA System to its three main competitors, the analysis proves that the system is a better product. Table 2-4 compares major features and prices of our device against its three main competitors: Accumetrics' VerifyNow System, Siemens' PFA-100 System, and Helena Laboratories' AggRAM Module and Analyzer.

While all of the products have an error detection system, our device's is more advanced since each channel has a twin channel running a test on the same concentration. This feature is very beneficial because it will allow errors to be easily displayed in the graphs that show the results for the twin channels. If there is a discrepancy between the graphs, the discrepancy could be accounted for by either a diode being broken or a channel being clogged or blocked. This error detection will verify if the test has to be rerun.

With a runtime of 5 minutes and an incubation period of 5 minutes, the MCCRA system also has a faster or comparable turnaround than the other products. The system that has the next fastest turnaround is Accumetrics' VerifyNow System which completes its test in 3 minutes and has an incubation period of 10 minutes. The product that has the longest turnaround time is Helena Laboratories' AggRAM Module, which is also the product that is used as our reference concept in the Pugh Matrix seen in the Product Concepts section of this report. While the device's runtime is only 3 minutes, its incubation time is 45 minutes because the whole blood sample needs to be centrifuged for 15 minutes and required to sit for 30 minutes before testing.

The testing procedure is very efficient. The MCCRA device uses the least amount of whole blood, 550 μL. Our microfluidic chip has eight assay channels that test four inhibitor concentrations so the patient's resistance to clopidogrel can be assessed in one test rather than two or three like other competing products. The procedure requires very little clean-up because all of the reagents and sample are kept on the chip. This is similar to the test cartridges for the PFA-100 System since all of the reagents are in this cartridge along with the blood once it is added. The VerifyNow and AggRAM Systems differ. The VerifyNow uses a test tube to mix all reagents with the blood before it is inserted into the machine and the AggRAM uses cuvettes containing the reagents and blood that are placed in the device.

Feature	MCCRA	VerifyNow	PFA-100	AggRAM
Runtime	5 minutes	3 minutes	5 minutes	3 minutes
Incubation Time	5 minutes	10 minutes	< 15 minutes	45 minutes
Sample Size	550 µL	2 mL	800 µL	900 µL
Testing procedure	8 assay channels 4 different assays	1 assay channel 1 baseline channel	1 assay channel	4 assay channels Up to 4 different assays
System Cost	$6,000	$8,000	$15,000	$22,990
Cartridge/Test Cost	$100	$50	$30	$70

Table 2-4 Competitive Analysis

Finally, our product is cheaper than its competition. The box and software, which are sold together as a package, have a list price of only $6,000 while the next cheapest device is $8,000. Helena Laboratories' AggRAM Module sells at a list price of $7,995 and its software costs $14,995 which means our device and software is roughly $17,000 cheaper. Our microfluidic chip is sold for $100 while rival companies have chips or kits that range from $30 to $70. We can justify that our chip is $100 because of the accuracy of the test. It is unlikely for the test to be run more than once while tests for the VerifyNow and the PFA-100 Systems will most likely have to be run more than once because the patient's dosage will need to be adjusted and re-tested.

2.9 Superior Product Concept

The MCCRA System has two main features that make it better than other competing products: advanced error detection and testing multiple concentrations on one chip.

The error detection system in the device consists of two channels for each inhibitor concentration. The duplicate channel will be located in a separate part of the channel bifurcation system in order to show multiple potential problems. The potential errors these channels check for are deformed channels from manufacturing complications and blockages in blood flow.

Another reason to have multiple channels is to verify that the visible light diodes are working properly. If the graphs from different channels for the same concentration vary, the diode could be malfunctioning, indicating a technical problem as supposed to a real result with incorrect clinical implications.

Testing multiple inhibitor concentrations at once on one chip is a very important advantage. This saves the hospitals time and money by measuring the patient's response to a range of concentrations and determining an 'effective dose' regime rather than a one-time parameter value. If a patient is in the hospital for only one test versus multiple, their hospital bed will most likely be open in a shorter amount of time which keeps the influx of patients for hospitals high and the hospital can make more money. Insurance companies and patients will also save money because they will not have to pay for multiple tests. The MCCRA System is creating a phenotype for the patient by testing various ranges of inhibition over a five minute time interval. It is superior to the competing systems because it does not just measure the patient's response at a single point. The MCCRA System eliminates the need for any guess and check procedures and allows for one single test, instead of two to three trials like its leading competitors.

The price of the system is also very comparable or lower than other competing products' prices.

2.10 Other Important Considerations

Safety is always an important consideration when running a company and producing a product. It is important for the product to meet the standards of the industry while also keeping the manufacturing floor safe for employees.

Material Safety Data Sheets (MSDS) for PDMS, collagen, MRS 2395, ADP, acetone, and isopropanol have been attached in the Appendix since all of these materials and reagents are used daily in our facility. Among the advantages of our process is the fact that there are no harsh or particularly corrosive or reactive chemical in use, both minimizing the risk to employees and the environmental impact of an accident.

Part 3 **Feasibility Stage**

3.1 Process Overview

3.1.1 Chip Diagram

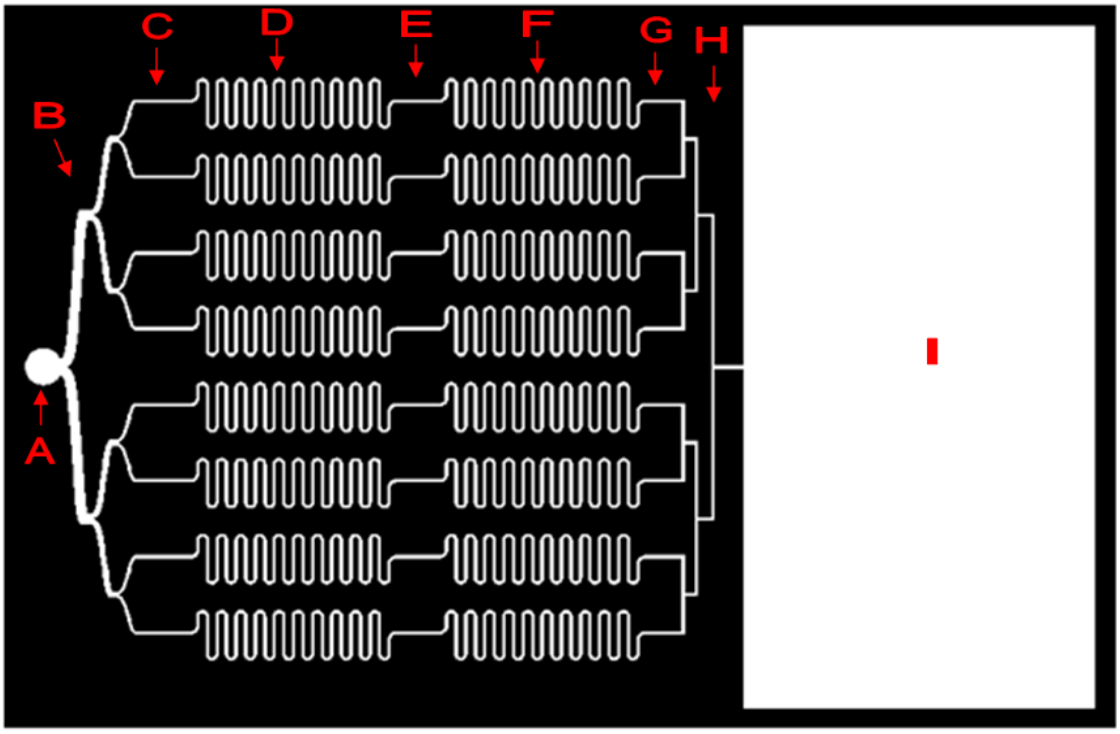
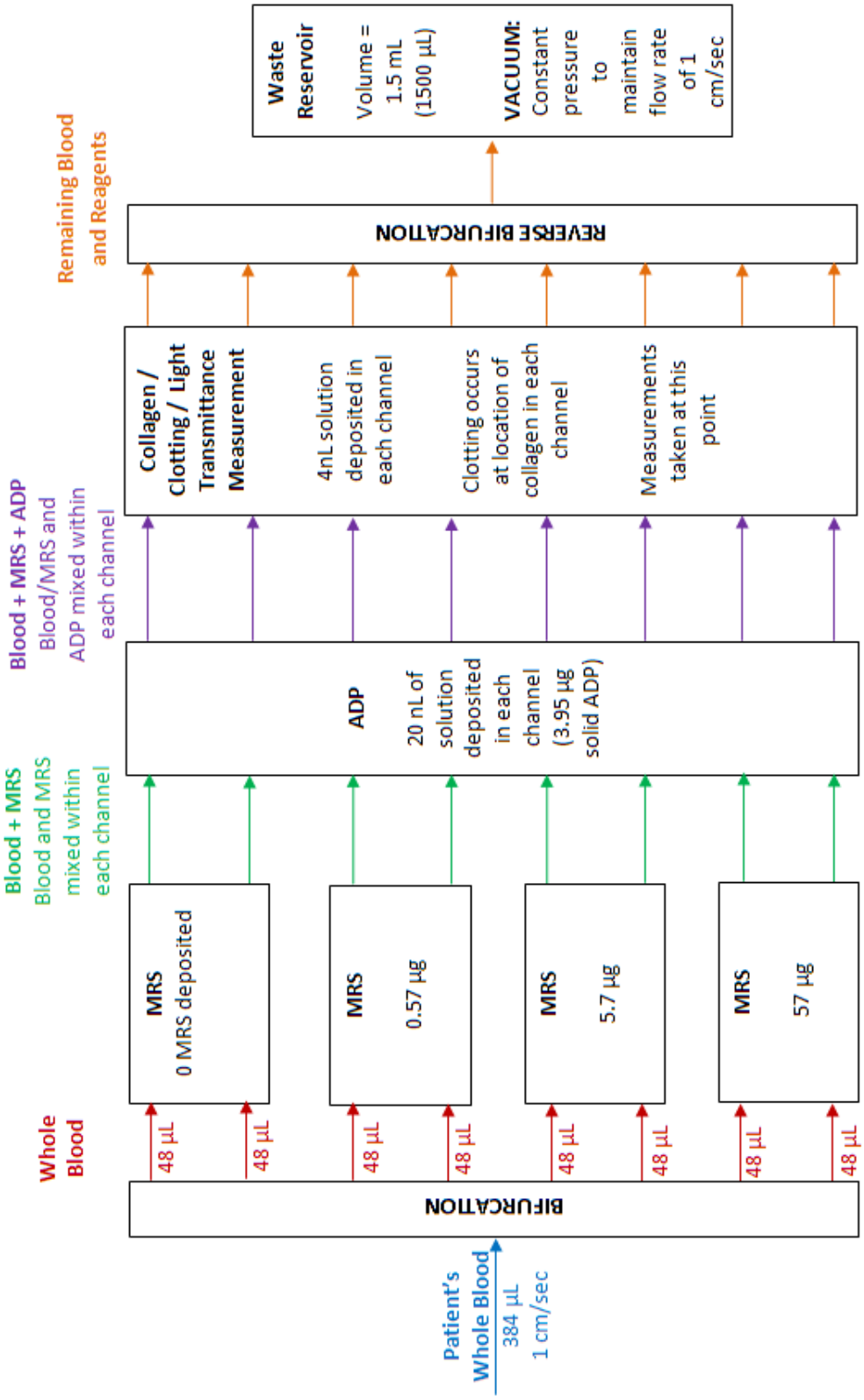


Figure 3-1 To-scale representation of the microfluidic chip (draw in CAD).
This image is what was used to pattern the photoresist mask used to create the PDMS chip.

Figure 3-1 is a CAD drawing of our microfluidic chip design. Point A is the location at which the patient's blood sample is injected onto the chip before testing. Point B is the bifurcation pattern used to create 8 channels that are equal in all dimensions, and arranged so that the flow through each of the eight channels is equivalent. At Point C, immediately following the bifurcation, the channels straighten out and this is where MRS 2395 is deposited. Point D is where the mixing section for the blood/MRS 2395 combination, including 10 turns and a series of grooves in the channels in order to make sure complete mixing occurs before reaching the next reagent. Point E is another straight area in the channels where ADP is deposited. At Point F, mixing is achieved for the blood/MRS 2395/ADP combination, again implementing 10 turns

and a series of grooves in the channel. The final straight section of the channels where collagen is deposited occurs at Point G. This is where clot formation will occur and light transmittance readings through the channel will be taken. Point H starts the reverse bifurcation of the eight channels back down to one to exit into the waste reservoir. This design is used in order for the blood to be pulled through the channels by the vacuum at identical flow rates, since no channel will have increased force applied due to its proximity to the vacuum. The waste reservoir is placed at Point I. The volume of this reservoir is approximately 1500 mL, which is more than 3 times the amount of space required to hold the blood sample. By providing sufficient volume in the reservoir to hold the entire sample, the blood will never leave the chip and enter into the device. Also, this should allow for enough space between the vacuum and the exit channel to make sure that none of the blood is sucked into the vacuum. This figure is the representation of the chip as it was fabricated to be used in lab tests (with the vacuum puncturing at the back center location of the reservoir). The scaled up manufacturing design, however, has the waste reservoir wrapping around one side of the chip and the vacuum puncturing the PDMS at the end of this section in order to further prevent the splattering of blood onto the vacuum.

3.1.2 Process Flowsheet



3.1.3 Flow through the Microfluidic Chip

3.1.3.1 Bifurcation

The patient's blood sample is injected into the chip at a designated location (See Figure 3-2). From this point, a single channel is branched into two equally sized channels. Each of these channels is then also divided in half. This is repeated so that there is a total of eight channels that are equivalent both in dimension and spacing (See Figure 3-2). For a more detailed description see Section 3.2.3.

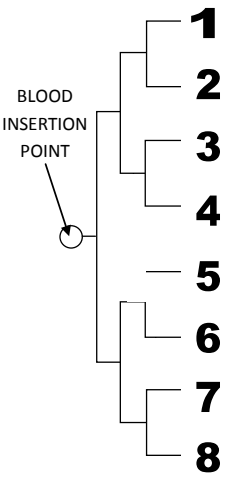


Figure 3-2 Bifurcation Depiction of the bifurcation of channels at the beginning of the chip in order to evenly divide the entry stream into eight streams. The point at which the blood is inserted in relation to the bifurcation is indicated.

3.1.3.2 Addition of MRS 2395

Once the blood has been equally divided into eight separate channels, it is flowed over dry MRS 2395. Each pair of channels has a different amount of MRS 2395. The four amounts of MRS 2395 used are 0 µg, 0.395 µg, 3.95 µg, and 39.5 µg. Having varying amounts of the compound allows for four sets of data, each corresponding to a different level of MRS 2395. Also, by having two streams contain each concentration, there is an automatic error detection system since the two values for each concentration can be compared for accuracy.

3.1.3.3 Mixing

After the blood has passed over the MRS 2395, it passes through a mixing section. In this part of the channel, the pathway turns 180° twenty different times (after each 0.05 cm length of channel) for a total of 20

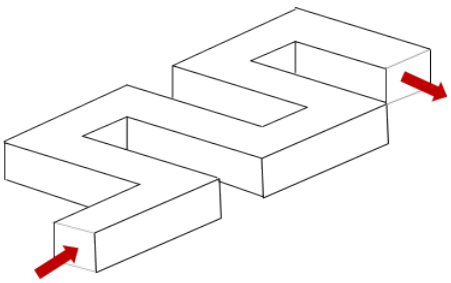


Figure 3-3 Three dimensional depiction of a section of one of the mixing portions of a channel. In this figure, there are three 180° channel bends. In each of the actual mixing sections on the chip there are 10 bends. The red arrows indicate the direction of blood flow.

full turns in order to aid in the mixing of the blood and the MRS 2395 (See Figure 3-3). In order to ensure total mixing, there are also grooves built into the top of the channels (See Figure 3-4).

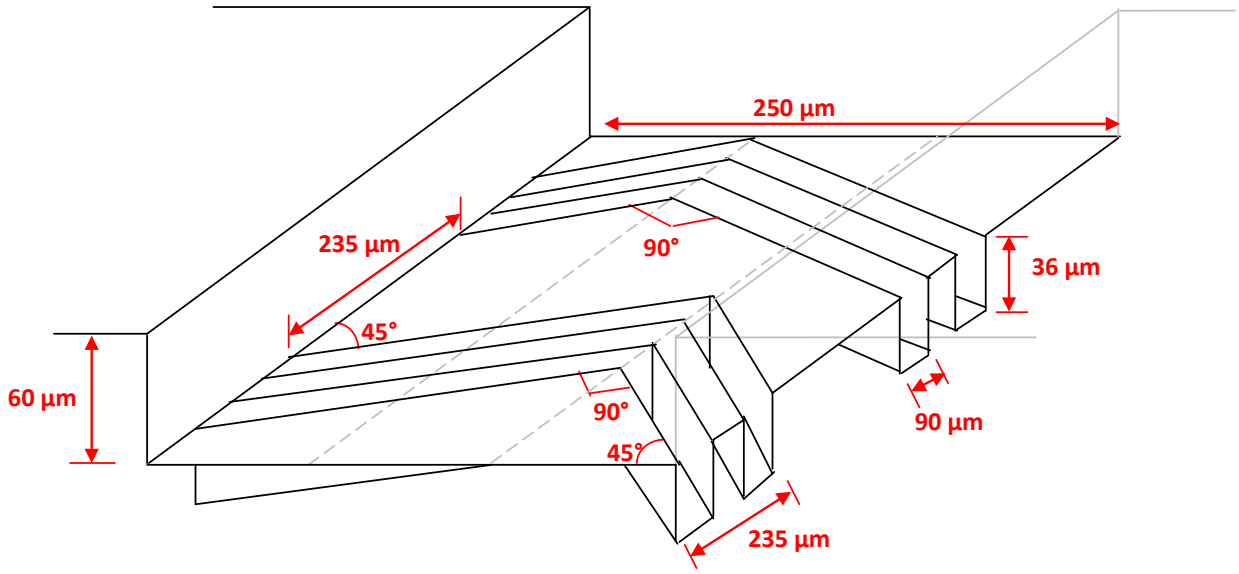


Figure 3-4 Three-dimensional depiction a section of mixing channel with grooves. (Not drawn to scale)
Note: this is a picture of the PDMS channel when fabricated, however, when joined with the glass surface, the PDMS is flipped upside down, and the grooves will be above the flow of the blood.

3.1.3.4 Addition of ADP

After mixing with the MRS 2395, the blood flows over a region containing an ADP deposit. The amount of ADP in each channel is 3.95 µg (equal amount in all eight channels). Following the same mixing mechanism as before, the blood containing MRS 2395 is mixed fully with the ADP.

3.1.3.5 Collagen and Light Transmittance

The blood containing MRS 2395 and ADP then flows over a surface coated with a collagen deposit of 4 nL (equal amount in all eight channels). As it passes over this region, the collagen activates the formation of a blood clot (See Section 2.1). Once the blood starts passing over the collagen, the MCCRA device will begin taking light transmittance readings (See Section

3.3). The amount of clotting that occurs will depend on the resistance level of the patient, as well as the amount of MRS 2395 that was added to each channel. (See Figure 3-5)

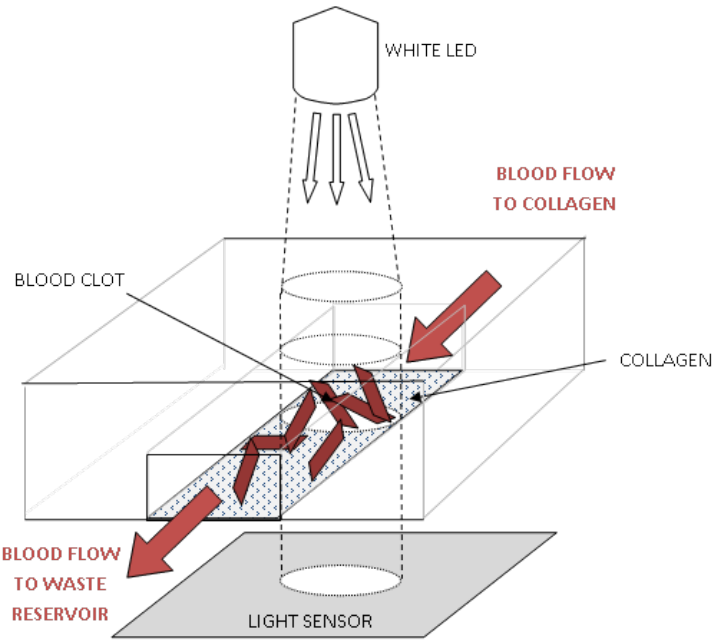


Figure 3-5 3-D depiction of the collagen section of one of the channels. The red arrows indicate the flow of blood through the channel. A blood clot forms on the collagen and a white LED located in the MCCRA device above this section of the channel shines on the clot. The magnitude of the transmission through the clot is measured by a light sensor located in the device, below the chip.

3.1.3.6 Waste Disposal

Once the blood has passed over the collagen, it proceeds through a reverse bifurcation setup and into the waste reservoir. The second bifurcation assures that the blood will be pulled at the same flow rate in each of the eight channels by eliminating a variance due to proximity to the vacuum. The waste reservoir is designed so that there is enough space to hold 1.5 mL of blood (approximately three times the 550 µL of sample that is used). By making the reservoir sufficiently large, all of the blood will be contained on the chip at all times, i.e. at no point in the testing process will blood reach the vacuum or any other component of the box. After the test is completed, the chip can be removed and disposed of without needed excessive cleaning of the box. Also, the vacuum is connected by a puncture into the top of the chip (through the PDMS layer) into the waste reservoir. By having the vacuum pulling from above the waste reservoir, the blood that settles into the bottom of the reservoir will be far enough from the vacuum that it will not be pulled up into it. (See Figure 3-6)

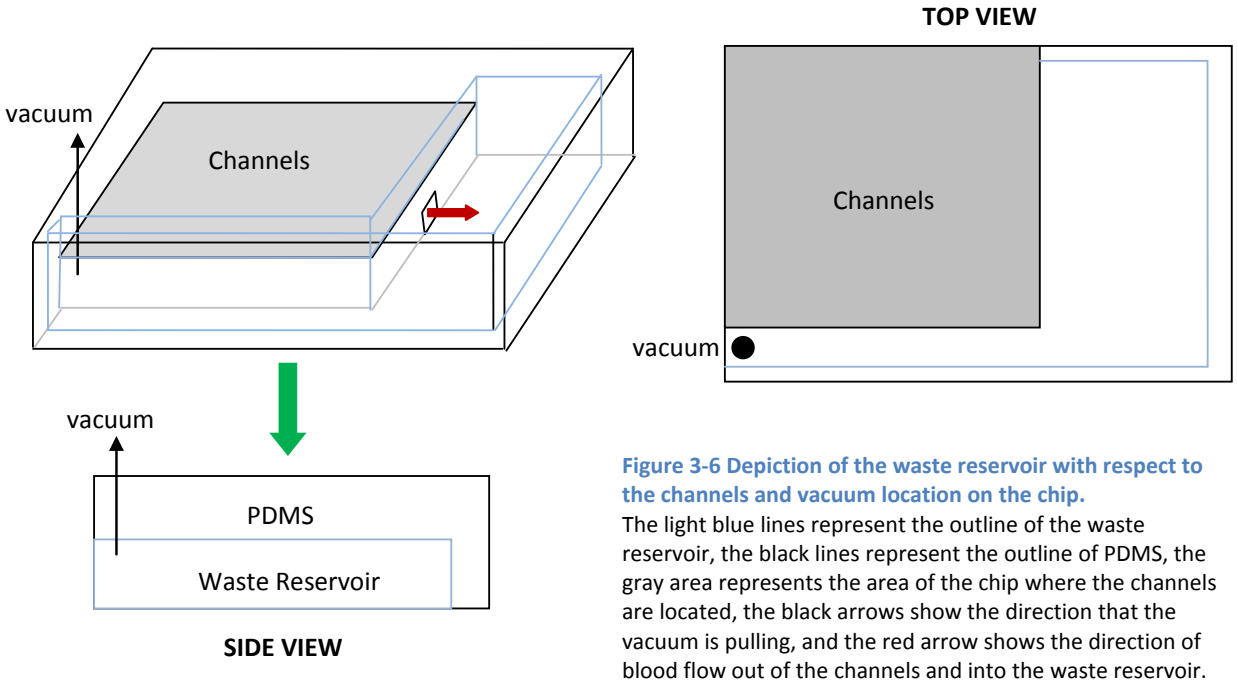


Figure 3-6 Depiction of the waste reservoir with respect to the channels and vacuum location on the chip.
The light blue lines represent the outline of the waste reservoir, the black lines represent the outline of PDMS, the gray area represents the area of the chip where the channels are located, the black arrows show the direction that the vacuum is pulling, and the red arrow shows the direction of blood flow out of the channels and into the waste reservoir.

3.1.4 Process Conditions and Reagent Volume

This section explains the unapparent process embedded between the blood entering the machine and read-out from the display. Shortly after 550 µL of blood enters the micro-channels of the chip, which is controlled at 37°C by a temperature reservoir within the box (See Box Design), it splits into 8 identical streams through graduated bifurcation. Each stream first encounters the MRS 2395 matrix and undergoes dissolution and mixing. Downstream to MRS 2395, the blood flow undergoes ADP-carbohydrate dissolution and mixing processes before flowing though collagen, where platelet aggregation is detected. The 8 streams are grouped into 2 identical sets of 4 streams. In either set, each steam dissolves a different amount of MRS 2395 to achieve 0X, 0.1X, 1X, and 10X the IC50 of 3.6µM. [24] Although the targeted concentrations of MRS 2395 are set and ready, the calculation that is needed to determine the amount of MRS 2395 deposition on the micro-channel to achieve such concentrations is rather involved. Table

3-1 gives a summary of the amount of depositions and detailed computation can be found in Appendix 7.2.

Reagent	Amt. in stream	Amt. deposit on chip
Whole Blood	550 µL	
MRS 2395 (0xIC50, 2 streams)	0	0
MRS 2395 (0.1xIC50, 2 streams)	0.36uM	0.00057 mg
MRS 2395 (1xIC50)	3.6uM	0.0057 mg
MRS 2395 (10xIC50)	36uM	0.057 mg
ADP (all 8 streams)	3.6uM	0.0057 mg

Table 3-1 Reagent target concentration in 8 flowing streams and amount (mg) for deposition on micro-channels.

3.2 Detailed Chip Design

3.2.1 Chip Fabrication

3.2.1.1 Manufacturing Considerations

The microfluidic chip for use in our point-of-care device will be manufactured in our own facilities. It was initially proposed that an outside company could be contracted to fabricate the chips, partly due to the large cost associated with constructing a fabrication facility. However, it was agreed that the intellectual property inherent in our device should not be put at risk by outsourcing the process.

As mentioned in an earlier chapter, the chip will be constructed of two layers: a bottom glass layer and a top PDMS layer. This patterned design must be present on the PDMS layer, whereas the deposition of the various reagents must occur on the glass layer. To pattern the PDMS, photolithography and soft lithography were both found as options. Advantages and

disadvantages were associated with both ways, and in the end a soft lithography technique known as replica molding was decided upon for use. Another issue that needed to be addressed was the method of deposition of the MRS 2395, ADP and bovine collagen. Use of a robotic pin tool was considered, as well as an inkjet dispenser for delivery. Since three reagents need to be deposited per channel and eight channels are present on one chip, 24 depositions must occur per chip. Therefore, the speed of delivery in each system was contrasted to insure that the goal of 4,000 chips manufactured per day could be attained. After considering a number of attributes, including price, a robotic pin tool was found to be suitable for our purposes. Finally, equipment including a curing oven, plasma oxidizer, and UV light emitter were needed for curing of the PDMS top layer, sealing of the two layers and sterilization of the device, respectively.

3.2.1.2 Patterning of PDMS Layer

The top layer of the microfluidic chip will be constructed with the elastomer known polydimethylsiloxane, or simply PDMS. This material was decided upon to form the foundation of the device because of its advantageous molding capabilities. Initially present in the form of a base and curing agent, a mixture of the two parts can be patterned with a certain design using one of any number of different methods: microcontact printing (μCP), replica molding (REM), microtransfer molding (μTM), micromolding in capillaries (MIMIC), solvent-assisted micromolding (SAMIM), and photolithographic patterning of photosensitive PDMS. All of these methods have advantages and disadvantages in their application to fabrication of our device.

Photolithography methods for fabrication of our device were first explored. Microfabrication in the electronics industry almost exclusively uses some form of photolithography due to its ability to use a single photomask for the mass-production of thin film patterned devices. Because of the rather large goal of one million chips to be produced per year,

a parallel process that could yield a high net output of devices per day while maintaining a high fidelity of the product was a desirable trait. Since photolithography has been used to great success in the electronics industry, a process that incorporated such was sought. The search resulted in the finding of a technique for making the PDMS layer of the device photosensitive, thus making it susceptible to patterning by ultraviolet light. The method called for the inclusion of benzophenone in the initial mixture of PDMS base and curing agent. Amounts of benzophenone to be included could range from 0.1%-15% (v:v). The addition of this compound led to the formation of a benzophenone radical when irradiated with UV light ($\lambda < 365$ nm), which abstracts a hydrogen atom from any suitable hydrogen donor. These radicals react with the silicon hydride groups present in the PDMS crosslinkers and prevent them from undergoing the traditional crosslinking reaction with the PDMS monomer. [25] Therefore, regions of the chip irradiated with UV light will be prevented from curing when heated, and the PDMS in these areas can be washed away with an organic developer (solution). In this case, the photoinitiator is termed a positive resist. The positive acting nature of benzophenone makes for a simplified fabrication process for the microfluidic chips. In addition, features on the order of a few microns can be easily fabricated using this method. Although the use of *photo*PDMS seemed like a viable option, a number of concerns were brought to our attention. First, the process was not entirely proven. The idea for the use of benzophenone as a photoinitiator and subsequent experimentation was carried out by a research group at the University of Cincinnati. While the results appeared promising and a number of papers have been published regarding *photo*PDMS, there is no industrial documentation showing the successful implementation of this idea into the fabrication of any elastomeric (PDMS) device. Furthermore, a number of the steps involved in treating the soluble portion of the surface might not be advisable. In the description given, the exposed

region was developed by dipping in a toluene solvent for about 5 seconds. The use of this organic solvent or any comparable solvent used as developer was advised against due to safety issues and FDA approval issues. [26] Consequently, another way to pattern the PDMS into our given design was required.

The field of soft lithography evolved from the need to produce desired patterns utilizing elastomers as a mold or stamp, as well as the requirements for a fabrication process that was low in cost, easy to learn, and easy to adapt to given set of circumstances. It possesses several advantages over conventional photolithography: soft lithography can utilize not just photoresists as a patterning surface but many different kinds of polymers, biological macromolecules and beads, it can pattern both planar and nonplanar surfaces in all three dimensions, and it can pattern features at about an order of magnitude smaller than photolithography. [27] A major key to soft lithography is the use of a single master to create multiple molds that can then be used to fabricate a large amount of devices. The master is normally made using conventional photolithography: resist is spun onto a silicon wafer, the image is imprinted on via irradiation with UV light or using e-beam lithography, the wafer developed and the pattern made. A PDMS mold is then made from this master, which in turn serves as the cast for the further fabrication of patterned PDMS layers that are used for any number of microfluidic chip devices. Elastomers are used as the material to make molds due to their ability to make conformal contact with surfaces over relatively large areas and the ease with which patterned layers can be released from the surface of such a mold. In addition to the advantageous qualities of PDMS such as low interfacial free energy, good thermal and chemical stability, and optical transparency to wavelengths as low as 300 nm, one of the most beneficial qualities it possesses is its durability. According to literature, PDMS molds were used up to fifty times without a noticeable decline in

performance. [27] For all of its positives, a number of potential issues exist that might become problematic in the future. First, the PDMS has been shown to shrink slightly upon curing. This would lead to a distorted patterned layer. [28] Also, due to the elasticity and thermal expansion of PDMS, fabrication of multiple layers or layers with nanoscale features might be difficult because of the trouble in accurate patterning across large swaths of area. Finally, the suppleness of the PDMS restricts the patterning of extreme aspect ratio features (very high or very low height/length) because of the risk of deformation. Despite some of these concerns, the usefulness and applicability of soft lithography to the fabrication of our microfluidic chip seemed too beneficial to ignore. Compared to traditional photolithography processes explored, soft lithography allowed for the production of many chips in parallel while retaining a simplistic design process. However, a number of different soft lithography fabrication methods exist. Each methodology differs in its fabrication of the mold that forms the template for the patterned layer. Thus, a detailed analysis of each was required to decide which fit the manufacturing of our device appropriately.

The first type of soft lithography method analyzed is termed microcontact printing (μCP). It is based on a fundamentally basic concept: the design on the surface of a PDMS stamp is exploited to form patterns of self-assembled monolayers on the surface of a substrate through physical contact. [27] Self assembly, which describes the change from a disordered state of material to an ordered state due to local, noncovalent interactions and not under any external pressures, is unique to microcontact printing. [29] In brief, the molecules of the system will keep changing states until the state with the lowest energy is found (chemical equilibrium). Furthermore the system will be at thermal equilibrium, which means that the system will spontaneously recover from any defects that might occur. Fabrication of a layer entails the

coating of a PDMS mold with a ligand (in the form $Y(CH_2)_nX$, where the X head group is commonly a methyl group and the Y anchoring group is sulfur, phosphate, etc.), which then comes in physical contact with the substrate. [27] Afterwards, etching or further deposition may occur to finish patterning the layer. This technique has the possibility of assisting us in creating the herringbone structure needed for proper mixing. However, the process appears too tedious and not amendable to a manufacturing process that demands high rates of production. Therefore, this idea was not investigated further.

The next method explored was microtransfer molding (μTM). In this method, a slim layer of liquid prepolymer (base plus curing agent) is applied to the patterned surface of a PDMS mold and the excess liquid is removed through any number of means. The combination of the prepolymer and curing agent within the mold is then put in contact with the desired substrate, when upon curing of the elastomer commences. After curing with UV light or heat, the result is a patterned layer of polymer that is left on top of the substrate. These patterned microstructures then may be used as masks to control the deposition or etching of the exposed substrate, or more simply as a mold for creation of other patterned layers. [30] The method can use a wide array of polymers as the mold materials, and fabrication of large structures (about 3 cm^2) can be completed in a small amount of time. These attributes seem to fit the requirements we are seeking in a fabrication scheme. However, two problems become apparent upon inspection. The first is relatively straightforward: our chip currently has dimensions of 4 cm by 6 cm and an area of 24 cm^2, whereas the maximum area of a device found in literature was only about 3 cm^2. Second, a layering problem exists if this fabrication mode is used. Our plans call for the bottom layer of our device to be a glass substrate, onto which the patterned layer of PDMS will make contact with. The channels and other patterned features must be present in this interface.

However, the product of microtransfer molding leads to a patterned surface not at the interface, but at the opposite side of the polymer layer. Although sealing of this side to a glass substrate coupled with subsequent removal of the initial substrate is possible, it seems like an unnecessary step. The use of a physical mold, though, seems to be a viable alternative to photolithography because of the ability to produce identical molds in high volume and combine them on one surface to fabricate many PDMS layers in parallel.

Micromolding in capillaries (MIMIC) is another soft lithography technique that was considered. In MIMIC, a mold composed of an elastomeric material is placed against a desired substrate. Channels intentionally formed between the mold and the substrate are filled with a low viscosity prepolymer by capillary action. The polymer is cured and the mold is separated from the device to yield a substrate layer with microstructures on its surface. [31] Again the range of material compatible with this technique is very broad, spanning UV- and thermal prepolymers to biological macromolecules. Additionally, the minimum feature size extends down to as low as hundreds of nanometers, far exceeding the spatial requirements of our device. However, the amount of time to diffuse through the channels by capillary action inhibits this technique by not allowing patterned layers to be fabricated fast enough to comply with the desired throughput. Also, relying on capillary action to fill the mold's channels might introduce significant error into fabrication and might lead to channel-to-channel variability between chips, and even between different channels on a chip. Finally, the amount of PDMS used would have to be large because the mold must have the channel features protruding from its surfaces, meaning capillary action would have to account for the deposition of most of the elastomer on the chip. Besides introducing further potential error and variability in fabrication due to the amount of fluid needed

to be moved, the patterned side would again be on the opposite side of the initial substrate-PDMS interface, leading to more unnecessary processing steps.

A fourth technique abiding by the tenets of soft lithography is solvent-assisted micromolding (SAMIM). SAMIM generates a pattern in the surface of a material using a good solvent that perturbs the material enough so as to make it pliable, but not to the extent of affecting the composition or stability of the mold. SAMIM proceeds by taking a PDMS mold and wetting its surface with the chosen solvent. [32] The mold is then placed onto the surface of the substrate. A thin layer of the substrate material is dissolved by the solvent, and this layer conforms to the shape of the mold. When the PDMS mold is released from the surface, the solvent evaporates and substrate solidifies. The surface of the substrate is now complementary to that of the surface of the mold. Solvent-assisted micromolding has been shown to work with a wide variety of materials, including organic polymers such as PDMS. Observations of submicron features patterned by this method showed very little defects. In the context of our device, a solid layer of PDMS would have to be made and then the PDMS mold plus solvent applied to pattern the surface. This patterning method fulfilled requirements relating to compatibility with PDMS, feature size and adaptability to a large-scale manufacturing scheme.

The last soft lithography method explored was termed replica molding (REM). The most basic of the subset of micromolding techniques, it allows the transfer of the information present in a mold to a layer of polymer by simple conformation to the template's shape. [33] Compared to other methods, large amounts of polymer are used in REM. The accuracy of information transfer from the mold to the surface depends on noncovalent interactions occurring within the polymer, the nature of the mold surface, and the speed at which the mold is filled with the polymer. However, the beauty of the process lies in its ability to create a patterned structure

complementary to that of the mold in just one step. The benefits this imparts to our manufacturing process are tremendous: since multiple molds can potentially be grouped together on one surface, many PDMS layers can be constructed in parallel. Furthermore, one master with the desired image translates into many molds, and the molds are made in simple and cost-effective way. Feature sizes with a resolution smaller than 10 nanometers were observed in laboratory experiments, which far exceed that needed on our microfluidic chip. [34] In addition, it has been shown that replication from a single master can proceed more than ten times without significant degradation in the quality of the patterned layer or mold. [27] Combined with the benefits of simple, straightforward micromolding, the durability of the PDMS molds makes replica molding the easiest soft lithography technique to transfer from benchtop to large-scale manufacturing.

After an exhaustive study of all options, it was agreed that our manufacturing scheme would take from the principles underlying replica molding. This micromolding technique satisfied our requirements of low cost, ease of production and ability to produce in high volume. However, the process would have to be custom fit to our needs. To achieve a chip production between one thousand to four thousand chips produced per day (depending on which production year considered), many hundred molds needed to be present on each manifold. It was decided that a few masters would be created, which would serve as the templates for the molds. The masters would be created from normal lithography means. Our design called for channels of several different lengths, with heights of 60 micrometers and widths between 250 micrometers and 1000 micrometers. The other major feature on the chip is the waste reservoir, which has dimensions of 1 centimeter by 2 centimeter by 0.5 centimeter. All of these features are far above the feature size limit of photolithography, and can therefore be fabricated with high accuracy.

With a silicon wafer as a substrate, master creation would begin by applying positive photoresist onto the substrate to give a resist thickness of approximately 250 micrometers. After exposure to UV light under the direction of a chrome mask that only permits passage of light in the areas that correspond to the channels and reservoir, the master is baked to cure the unexposed resist while the exposed resist is washed with an organic solvent (developer). The result is a substrate with 250 micrometer deep channels and reservoirs in positions corresponding to those on our chip. However, all of the channels need to be 60 micrometers in depth and have a herringbone structure that is recessed 36 micrometers into the channel floor. Therefore, a second deposition of approximately 190 micrometers of positive photoresist is needed. The master is then exposed to UV light according to the layout of another mask, which will have openings for the herringbone structure in the proper channels. After curing and developing the master, channels of 60 microns and the mixing features of 36 microns are patterned. Finally, the master is silanized to give the patterned surface a hydrophobic nature, which reduces any adverse

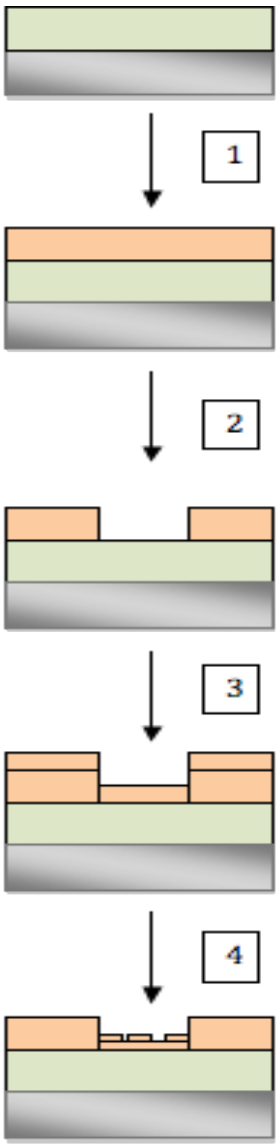


Figure 3-7 The accompanying diagram shows the processing of the master that will be used to construct the PDMS molds.
A silicon wafer (grey) with oxide (green) deposited on it is spin-coated with a positive resist [1]. The resist is patterned to give 250 micron deep wells [2]. Another layer of positive resist is spin-coated onto the wafer [3]. Finally, the resist is again exposed to UV light to fabricate the herringbone structure at the bottom of the channels.

reactions or absorption. An outside microfabrication facility would be contracted to for master creation because of the large cost associated with buying the necessary photolithography machinery that would only be operated sparingly.

When the master was finished, PDMS molds complementary to the surface pattern of the master could be made. As mentioned prior, parallel production of the PDMS layer of the microfluidic chip called for large manifolds containing hundreds of molds. PDMS was chosen as the mold material because of several reasons. First, PDMS is durable enough to withstand repeated use as a stamp and not become structurally compromised. Studies showed highly accurate pattern transfer by PDMS stamps after more than ten uses. [(34)] Since our molds will only be used once or twice per day, their lifetime could be on the order of a month or two. Also, polymers patterned against the PDMS mold won't adhere or chemically react to the mold's surface. The only concern is that the aspect ratios of the channel features (the features would extrude from the mold since its pattern is complementary to that of the master and final PDMS layer) fit into the range allowed for PDMS molding. Specifically, the channels all have aspect ratios (a ratio of height/width) that fall somewhere between 0.06 and 0.24, while the reservoir has an aspect ratio of about 0.125. One study found that aspect ratios of features should fall between 0.2 and 2 to obtain defect-free molds. [(35)] Since our features fall on the low end of that scale, the integrity of the pattern of our PDMS molds could be in question. However, small changes to our chip design could be undertaken during the initial startup of our manufacturing to correct any problems with information transfer by our mold. It was decided that production of the molds and manifolds would be outsourced because of the cost of excess equipment necessary for their construction, as well as the only occasional need for a new manifold (about once every month or two, depending on the actual durability of the PDMS mold). The manifolds will be

filled with PDMS and thermally cured, giving the desired PDMS block with channels and reservoir ready to be sealed with the bottom layer. With a successful way to fabricate the PDMS layer within our manufacturing scheme found, the deposition of reagents is considered.

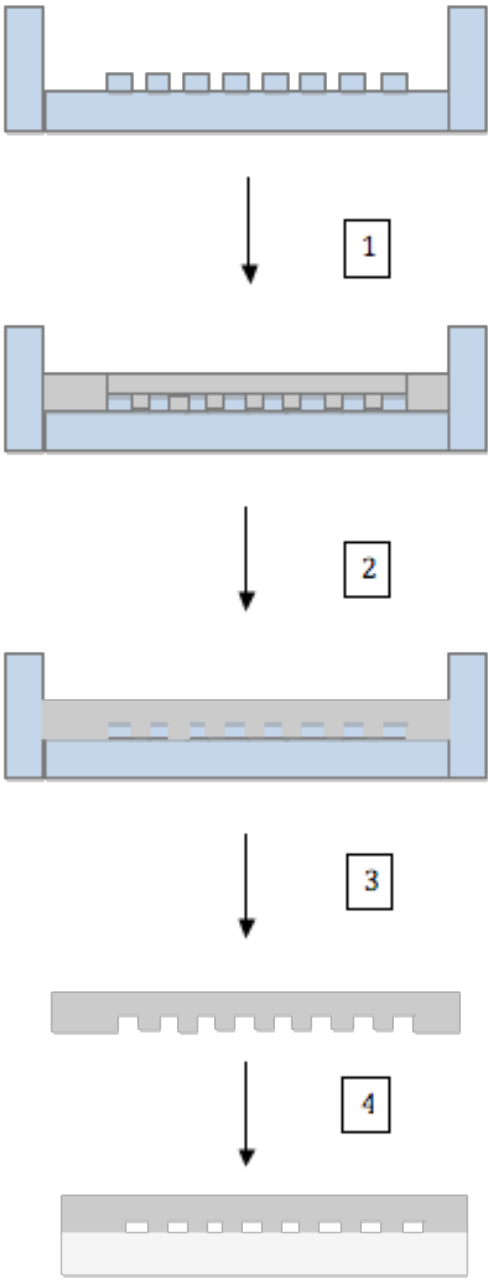


Figure 3-8 The accompanying diagram shows the fabrication of the PDMS layer from its respective mold. The mixture of PDMS base and curing agent is applied to the PDMS mold at a volume consistent with the desired chip features [1]. The mold is then cured for 2 hours at 80°C [2]. Due to low interfacial energy, the PDMS layer is easily plied away from the mold [3]. The PDMS mold is now free to be used again, while the PDMS layer and glass substrate are surface treated and subsequently bonded together [4].

3.2.1.3 Deposition of Reagents on Glass Substrate

To successfully carry out an assay of the degree of clopidogrel resistance expressed by the patient's blood, three reagents must be present on the chip. The first is collagen, which acts as a substrate for the deposition of platelets and as a secondary activator. ADP, which acts to stimulate platelet aggregation, also needs to be deposited. Finally, the platelet aggregation inhibitor MRS 2395 is deposited on chip in different concentrations. Since the volumes to deposit will be in the tens to hundreds of nanoliters range, normal liquid delivery systems will not suffice. Technologies that have evolved from drug discovery allow the addition of nanoliter to picoliter amounts of reagent in order to test large libraries for compounds that inhibit a biological target. Two fluid delivery systems seem to be candidates for use in the manufacturing of the microfluidic chip: inkjet fluid handling and robotic pin tool delivery. The mechanics behind each system and the ability to fit our requirements were explored.

3.2.1.4 Inkjet Fluid Handling System

A ubiquitous technology vital to the operation of the personal computer printer, inkjet fluid handling is a valuable technology because of its ability to deliver very small volumes of reagent. Fluid is delivered in two distinct modes: continuous and demand. In continuous mode, fluid flows through a small opening while an electrochemical device creates pressure oscillations that cause the fluid to break into droplets of roughly equal size. Once past this opening, electrostatic charge is induced in the drops by the action of an electric field. Another electric field is then used to direct the drops toward a surface. Using this mode, droplets ranging from 10 ficoliters to 0.5 microliters are delivered between 80-100 times per second. [(36)] If the machine is run in demand mode, a potential across a layer of piezoelectric material leads to the formation of

a pressure change in the fluid. The result is the discharge of a single droplet to the desired substrate. Therefore, this mode is much more deliberate in its application.

Our process required deposition volumes somewhere in low nanoliter range, which is easily achieved by this device. Additionally, our goal of 4,000 chips manufactured per day meant that the time to deposit the reagent per channel should be as fast as possible. The most optimistic estimate of deposition time was found to be about one site every 1-2 seconds. To accomplish our manufacturing goals, though, much more than that was needed. Not only are there 8 sites per chip, but three different reagents needed to be deposited in eight places, adding up to a total of twenty-four spots. Using the optimistic deposition rate of five to ten sites per second, it would take about two seconds to deposit one reagent on a chip. The total time to complete deposition on one chip would probably be between six to eight seconds. While this is not exactly detrimental to the manufacturing process, there is an obvious need for multiple printheads to realize our daily manufacturing goal. Initial pricing of an inkjet fluid handling system approached almost $200,000. Assuming the prior rate of deposition, we would need three separate systems to complete fabrication of 4,000 chips per day. To pay almost half of one million dollars just on the fluid delivery system seemed like an exorbitant cost. Therefore, analysis of inkjet fluid handling systems was halted and the potential of using a robotic pin tool for liquid transfer was investigated.

3.2.1.5 Robotic Pin Tool

Known for its use in the screening of compound libraries in drug discovery, robotic pin tools are commonly used in the transfer of small reagent volumes between a stock solution and a substrate. Fluid is transferred by simply adhering onto the pin tool by surface tension and then coming in contact with the substrate surface. The pins that actually transfer the liquid are arrayed

in a head that contains a certain amount of pins. For our deposition, a standard 384 pin tool head could be used. This gives about a 4.5 millimeter space between pins, which should insure that we can deposit the reagent accurately. The pins that will be used are hydrophobic/lipophobic coated, which discourages reagent adsorption. 4 nanoliter, 10 nanoliter and 20 nanoliter pins would be used for the deposition of collagen, MRS 2395 and ADP, respectively.

When a robotic pin tool system with these specifications was priced, the cost to implement one was found to be less than half that of the inkjet fluid delivery system. Specifically, a mounting plate for the device cost $375, the pin tool head cost $2,521, the pin tools themselves cost either $9 or $11 per pin depending on the delivery volume, and the robotic station cost $20,000 (all prices were of Beckman equipment). The total here comes to about $30,000, assuming a large amount of pin tools are bought. Although the price may not be 100% accurate, the money invested in such a system is considerably less than inkjet system and allows us to invest in multiple robotic pin tool systems to increase parallel production and throughput of the microfluidic devices. No concrete evidence was found regarding deposition rates, but a value of 3 seconds per transfer was used. The robotic pin tool system from Beckman comes customizable, which is useful because of the need for adaption to a manufacturing line.

3.2.1.6 Reagent Loading Scheme

Contrasted against each other, the decision to use the robotic pin tool for deposition of the reagents was a straightforward one. First, the robotic pin tool could deliver all eight deliveries of the same reagent in one deposition. Although some calibration of the system and alteration of the chip design might have to occur, the spacing should allow for 8 pins to be present on a pin head and deliver the reagent to the desired position. The other main difference was the estimated cost of the systems. With purchase costs differing by almost $400,000, the much lower cost

investment possible with the robotic pin tool system lends itself to the purchase of multiple systems. This benefit helps us realize our goal of 4,000 chips fabricated per day, and allows for a smaller expenditure on equipment if production was to increase in the future.

One of the only concerns with the deposition of our reagent is exactly how much will evaporate. With such small volumes and large surface area to volume ratios, evaporation occurs quite quickly after the droplets are applied to a substrate. In literature, an estimate of 1 nanoliter of aqueous solution will evaporate in approximately thirty seconds. [37] Therefore, the amount of time it takes to deposit the reagents and then seal the PDMS and glass substrate is crucial to the extent of evaporation that will occur. Additional steps can be taken to reduce evaporation of the reagents. Including viscous substances, such as glycerol, in the reagent mixture is shown to reduce evaporation while not reacting with the deposited compounds. The temperature can also be maintained low enough such that the vapor pressures of the compounds are decreased, leading to less spontaneous evaporation. Finally, the manifolds can be temporarily sealed following deposition to further reduce any loss of reagent. It will probably take a combination of the following precautions to combat evaporation and maintain the reagent's concentration on chip. The problem will have to be addressed with appropriate design changes during the first year of design.

3.2.1.7 Curing Oven

Once the PDMS is deposited into its mold, the prepolymer and curing agent need to be allowed to set. This process crosslinks the polymer and forms the basis for its solid support. Our recipe calls for the PDMS to be cured for two hours at 80°C. Initial plans required two batches of 2,000 PDMS molds each. Using a rough estimate of actually area of 2,000 PDMS molds plus more area in between the molds and on the edges, the size of the manifold was determined to be

about 55 ft^2. After contacting a number of companies, two ovens were found. The first oven found was from Engineering Production Systems. For an inside chamber area of approximately 75 ft^2, and with outside dimensions of 6 ft x 5.5 ft x 7 ft, one Composite Curing Oven from EPS would cost us about $50,000. The oven is fairly high technology, especially considering our very basic needs and high tolerance. Another oven, from Wisconsin Oven Corporation, was priced at $22,400. The inside area of this oven was about 60 ft^2, which is slightly smaller than the EPS oven but should accommodate our needed size. Since both are equivalents in the eyes of our process, the SWN 610-6 oven from Wisconsin Oven Corporation was chosen for its significantly lower price. [38]

3.2.1.8 Plasma Oxidizer

To irreversibility bond the two layers together, the adjoining surfaces need to be treated by plasma oxidation. This is accomplished by a plasma oxidizer, which is a machine that converts gas into ions and directs them at a substrate. PDMS is a repeating polymer consisting of $–O–Si(CH_3)–$ subunits. However, introducing the surface of PDMS to air plasma leads to the replacement of the silane moiety with a silanol group. When this polar surface comes in contact with another substrate, the hydroxyl groups of the silanol condense with hydroxyl, carboxylic or ketone groups from the substrate to form a seal. [39] The bonding between the glass substrate and PDMS layer in our device yields an inorganic ester bond (Si–O–Si) concomitant on the loss of water. The covalent relationship between the surfaces is manifested by the irreversible bond that forms between both surfaces. A surface treatment machine from Electric-Technic Products accomplishes this task. The BD-80 Corona Treater, priced at about $5,000 per unit, is capable of treating one surface with air plasma within the desired 1 minute window. An adaptor kit made it

possible for the machine to be hooked up to a conveyor belt for repeated surface treatment of many layers. Thus, it was decided that the BD-80 Corona Treater would be purchased.

3.2.1.9 UV Sterilization

As a precaution, it was advised by a number of consultants to sterilize the microfluidic chip before storing it to insure no contamination issues arose. Therefore, after packaging into vacuum-sealed plastic bags, it was decided the device would pass under an array UV lamps to insure a sterilized environment in the chip. A lamp from Cole-Parmer, the UV Germicidal Lamp EW-97505-05, was chosen for the task. An array of 5 lamps would be put on the end of each production line, and the packages would simply pass under them on their way to refrigerator storage.

3.2.2 Chip Manufacture

3.2.2.1 Microfluidic Chip Recipe

Thirty minutes prior to the beginning of each day, PDMS prepolymer will be mixed with viscous curing agent in a large tank. Needing 17.49 millilters of total PDMS in each chip, assuming less than 1% shrinkage, and abiding by the 10:1 (v:v) ratio of base to curing agent, the tank will contain about 70 L of PDMS. It will be mixed with an impellor at low RPMs for approximately 10 minutes.

Two manifolds containing 2,000 molds each will be used for the curing stage. Each manifold will have two sets of valves with ten spouts each. These will deposit 17.49 mL into each mold, taking approximately 20 minutes. Both manifolds will then be placed in the curing oven. Currently, the curing time is estimated to be about two hours.

As the PDMS layers are being cured, reagent stocks for each robotic pin tool system will be prepared. 10 microliters of bovine collagen will be added to the refrigerated reagent store of each robotic pin tool system. 30 microliters of 200 mM ADP will then be added to the reagent storage of each robotic pin tool system. *In vitro* testing needs to be done to ascertain the amount of MRS 2395 needed per chip, but an estimation of 20 microliters for the reagent store will be used until then.

Deposition of collagen, ADP, and MRS on the glass substrate, which will be carried out by three separate 384 well pin heads, should take 3 seconds each for alignment and actual deposition of the liquid. This leads to a total deposition time of about nine seconds. Also, the depositing of the reagents will all take place in a slightly cold environment to protect against rapid evaporation.

When the curing bake of the PDMS is complete, the PDMS layer will be placed into smaller manifolds containing 100 each. Robotic arms that have suction devices on their tips will carry out transfer from a large manifold to a smaller one. The smaller manifolds will then progress down the conveyor belt to meet with the finished glass substrate for surface treatment.

With two surface treatment devices working in parallel, the surface of the PDMS layer is oxidized in one minute and then brought into contact with the glass substrate for another 30 seconds. With the microfluidic chips now in one manifold, the resulting 20-chip manifold is placed onto the conveyor belt.

The devices are taken from the manifold via a robotic arm and placed in a packaging machine that seals the chips in a plastic pouch. As the chips are transported to our freezer, they

pass through a UV light array, which effectively sterilizes them for their lifetime in the packaging.

As mentioned prior, the goal will be to fabricate approximately 4,000 chips per day, which leads to a yearly manufacturing goal of one million microfluidic chips per year. In the first two years of production though, the production goal will only need to be about 200 chips per day. It is during this time that many chips will be needed for late-stage concept testing, quality assurance and manufacturing process testing, and, most importantly, clinical trial testing for FDA approval. Therefore, the majority of the chips produced during this time will not be for sale but for testing (See Section 4.1).

3.2.2.2 Manufacturing Schedule

The full U.S. market is assumed to be 1.1 million chips per year, which is equal to the number of Percutaneous Coronary Intervention (PCI) procedures performed in the United States every year. This number was used to determine the total market since the main use of the MCCRA system is going to be before PCI procedures, when administration of clopidogrel is required. Since there are other similar products already on the market, 100% market capture is unrealistic (See Section 2.8). For the basis of calculations, it is assumed that the total market captured by this product is 50%, or 550,000 chips per year.

Several factors are considered and several assumptions are made in the determination of the number of chips that must be manufactured per day in order to meet this market capture goal. The first assumption is that the manufacturing facility will operate 8 hours per day, 5 days per week, and 50 weeks out of the year (250 days total). The number of chips needed for sale is determined by Equation 3-1.

$$\frac{550{,}000\ chips\ per\ year}{250\ days\ of\ operation\ per\ year} = 2200\ chips\ produced\ per\ day \qquad (3\text{-}1)$$

The next assumption that is made is that approximately 25% of the chips that are made during a manufacturing day will be used for quality assurance testing and cannot be counted towards the chips to be sold. Therefore, in order to produce 2200 chips to be sold, 2934 chips must actually be manufactured per day (See Equation 3-2).

$$successful\ chips\ made\ per\ day * 75\% = 2200\ chips\ produced\ for\ sale \qquad (3\text{-}2)$$

The final assumption that is made is that 25% of the chips that are manufactured will fail due to defect. Therefore, in order to create 2934 successful chips, 3912 chips must be produced (See Equation 3-3).

$$total\ chips\ produced\ per\ day * 75\% = 2934\ successful\ chips\ produced \qquad (3\text{-}3)$$

(Note: for ease of calculations for manufacturing and financial analysis, this number was rounded to 4,000 chips manufactured per day).

Based on the procedure for creating the chips, a manufacturing schedule was created (See Figure 3-9).

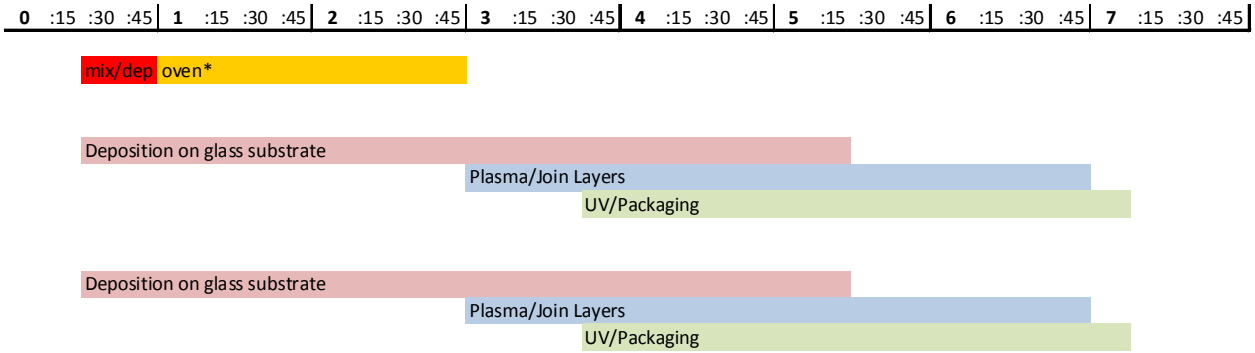


Figure 3-9 Schedule of a single day of operation for manufacture of the MCCRA chip. The numbers across the top represent the hour of operation (in bold) and the breakdown of each hour into 15 minute increments. Assume that hour 0 = the beginning of the day. Each colored band represents the length of time required for a specific step. Bands separated by a space represent steps that occur simultaneously, but independent of each other (i.e. what is done in one band does not have any effect on the other). Bands that are layered on top of each other also represent steps that are occurring simultaneously, however the products of each step are used in the next, and therefore the completion of one is dependent on the step represented by the band directly above it.

This schedule is based on an eight hour day of manufacturing (time 0 =9:00 AM), assuming 30 minutes of time prior to operation for opening/setup of the facility and 45 minutes after completion of manufacturing for cleanup/shutdown. The red box labeled "mix/dep" represents the time required for the mixing and deposition of the PDMS onto the molds. Mixing takes approximately ten minutes, and each deposition is estimated to take 3 seconds. Since the deposition device has the capability of depositing 10 chips at once, the time for this step is determined by Equation 3-4.

$$\frac{4{,}000\ chips * 3\ seconds\ per\ depostion}{10\ chips\ per\ deposition} = 20\ minutes \qquad (3\text{-}4)$$

Once the PDMS has been deposited, all 4,000 chips are put into the oven, for a total curing time of 2 hours. Before going into the oven, the chips are placed onto 20-chip manifolds (See Figure 3-10) for easier movement of chips between steps. The oven (Wisconsin Oven Corporation, Model: SWN-610-6) has internal dimensions of 6'x10'x6'. [40] The dimensions of the base allow space for 3 manifolds by 4 manifolds (12 total manifolds). In order to fit all 200 manifolds, 17 shelves will be required for inside the oven, with the shelves situated approximately 10 cm apart.

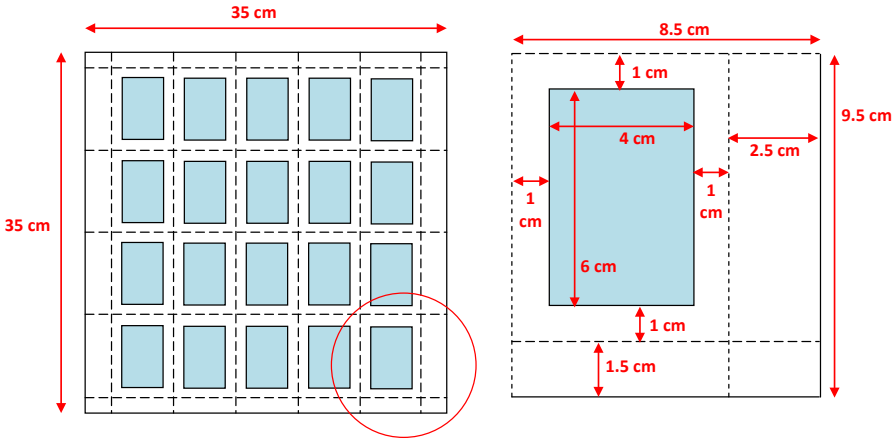


Figure 3-10 Manifold for chip manufacturing. The image to the left shows the manifold layout, consisting of 20 total chips. The image to the right is an enlarged depiction of the section of the manifold that is circled in red on the upper picture with dimensions labeled. The white area represents the tray holding the chips and the blue rectangles represent the chips themselves.

While the PDMS molds are in the oven, deposition of the reagents onto the glass substrates will be taking place. It is estimated that deposition will take 3 seconds per site. Since there are 3 reagents in each of 8 channels, there is a total of 24 sites that need to be deposited on each chip. This would results in a total of 80 hours of deposition if each site was deposited one at a time. In order to cut down on this time, the deposition device will be equipped to deposit each reagent in all eight channels at once. This will instead take 9 seconds per chip, for a total of 10 hours (see equation 3-5).

$$\frac{3\ seconds}{reagent} * \frac{3\ reagents}{chip} * \frac{4{,}000\ chips}{day} = 10\ hours \qquad \textbf{(3-5)}$$

Although this reduces the time required for deposition, 10 hours is still too long to fit into a normal manufacturing day. Therefore, two deposition machines will run in parallel, reducing the time required for deposition in half to 5 hours.

Once the PDMS layers have been removed from the oven, plasma oxidation and sealing to the glass substrate can begin, using those substrates that have already been deposited. The plasma oxidation/joining to glass substrate step for each manifold is estimated to take approximately 2 minutes, then an additional 30 minutes to sit and finish the binding process. Since each manifold can be oxidized while the previous begins the 30 minute resting step, the total time required for this part of the process is estimated at 8 hours. Again, this step will be divided in half and run simultaneously, reducing required time to 4 hours. Once the PDMS and glass layers have been joined and have rested for 30 minutes, the UV sterilization and packaging step can begin. The total estimated time for this part of the process is 3.5 hours (for half of the chips, again run simultaneously).

Once the packaging is complete, the chips are placed in a large refrigerator for storage until needed for shipment or quality assurance testing. As shown in Figure 3-9, many of the manufacturing steps can overlap, saving time, allowing for the manufacturing of 4,000 chips to be completed during the 8 hour work day.

3.2.2.3 Manufacturing Facility Floor Plan

Taking into account the order of manufacturing as well as the general dimensions of the required equipment, a basic floor plan was laid out (see figure 3-11).

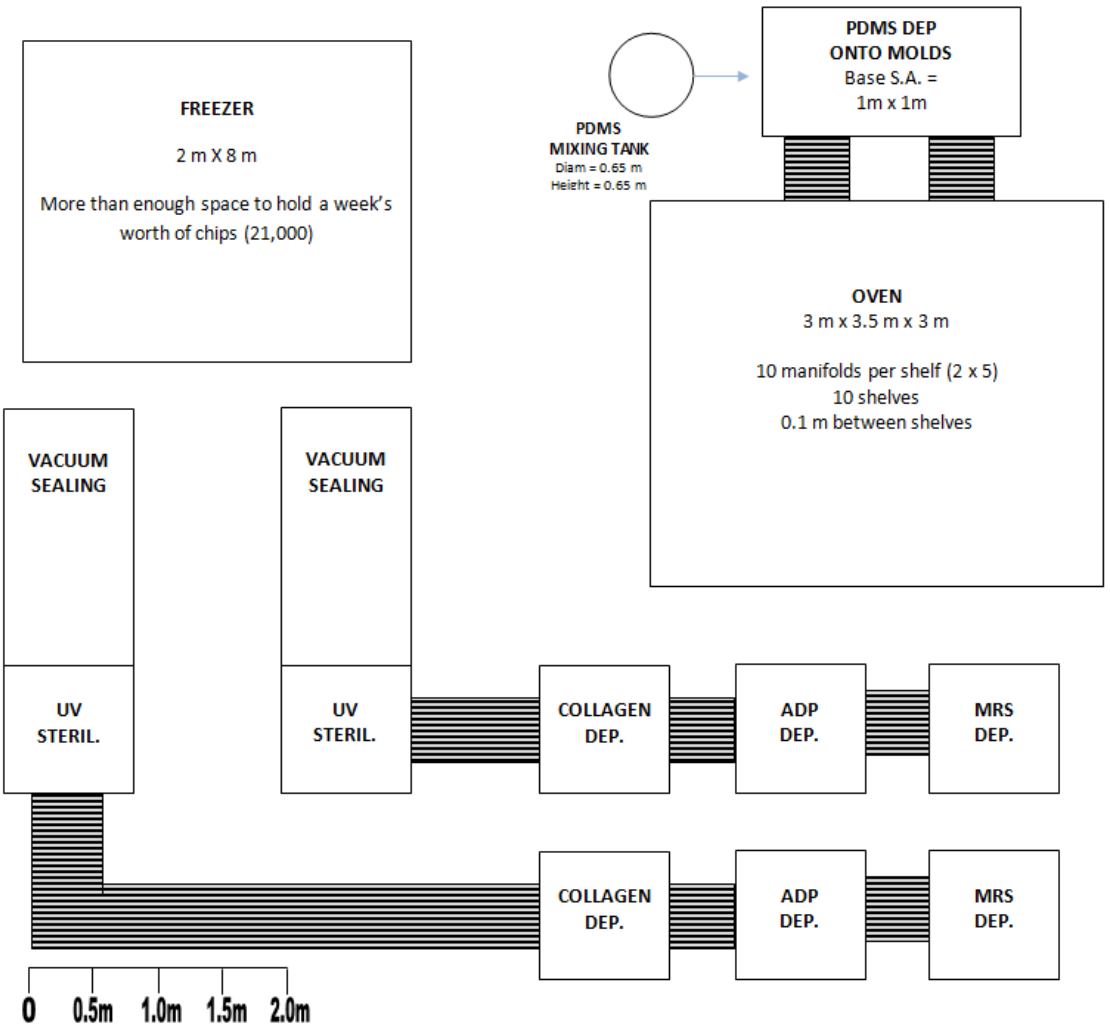


Figure 3-11 Basic Floor plan schematic for the chip manufacturing facility.
It is based on the order of the steps in the process and the dimensions of the equipment to be use. Note: Size conversion scale is located below the diagram to the left.

3.2.3 Channel Dimensions

To ensure complete mixing and fully developed flow, bifurcation is used to split the inlet stream into eight equal streams. Murray's Law is followed to calculate the effective diameter (d_{eff}) of the parent channel (r_p), or the channel that splits into two channels to form the daughter channels (r_d). Murray's equation is shown below in Equation 3-6.

$$r_p^3 = r_{d_1}^3 + r_{d_2}^3 + \cdots + r_{d_n}^3 = 2 * r_d^3 \quad \textbf{(3-6)}$$

The path-length (l) of each branch before the next separation should five to six times the effective diameter of the micro-channel.

$$\boldsymbol{l = 5 * d_{eff}} \quad \textbf{(3-7)}$$

The inlet channel has a width of 1 mm and after bifurcation our eight channels have the width of 250 µm. All channels have a height of 60 µm with the exception of the waste reservoir well which is .25 cm high. The final lengths and diameters of the channels are shown in Table 3-2. To see all of the calculations for the channel widths and length, please refer to Appendix 7.3.

Channel	Width (µm)	Height (µm)	Effective Diameter (µm)	Entry Length (µm)		Entry Length (mm)
Final 8 Channels	250	60	138	691	=>	0.691
4 Channels After 2nd Split	397	60	174	871	=>	0.871
2 Channels After 1st Split	630	60	219	1097	=>	1.097
1st Channel	1000	60	276	1382	=>	1.382

Table 3-2 Channel Dimensions

3.2.4 Flow Characteristic

It has been shown that shear rate at the vessel wall is an essential factor in blood clot formation both *in vivo* and *in vitro*. Flow velocity is positively correlated with shear rate given

the dimensions of the micro-channel. In our chip design, the fluid flow rate is controlled such that average shear rate at the channel wall is comparable to the *in vivo* shear rate, which is about 1000 s^{-1} in human arteries. With the channel dimension specification (w=250μm, h=60μm) and the assumed physical properties of water for whole blood[2], the 3D velocity profile can be solved. (See Appendix 7-11) The shear rate is defined by the following mathematical relation:

$$\dot{\gamma} = \frac{v}{h} \qquad \textbf{(3-8)}$$

where v(x, y) is a function of position of the cross-sectional plane and h is the distance from the channel wall.

From the velocity profile, the fluid velocity required to achieve an average shear rate of 1000 s^{-1} is then computed to be 0.01 m/s or 1 cm/s.

Fluid flow is generally characterized by the Reynolds number (Re), which is a ratio of inertia to viscosity. The dimensions of the microfluidic platform constrain the fluid flow to the laminar regime, typically, with Reynolds number close to 1.

$$\boldsymbol{Re = \frac{uD}{\nu} = \frac{\left(1\times\frac{10^{-2}m}{s}\right)\times 9.68\times10^{-5}m}{6.84\times10^{-7}\,m^2/s} = 1.415} \qquad \textbf{(3-9)}$$

where D is the characteristic length (in this case the hydraulic diameter of the channel cross-section) and $\boldsymbol{\nu}$ is kinematic viscosity, assuming the physical properties of water.

The small Reynolds number implies that the flowing stream in the microchannel lacks turbulence that is normally required for rapid mixing; this is one of the key limitations confronting our chip design. To overcome the sluggish nature of this type of flow and accelerate

[2] Blood plasma is 90% water. Therefore this is a reasonable approximation

mixing, a special structure has to be incorporated to the channel. Discussion on this issue will be continued in the following sections.

3.2.5 Controlled Dissolution of the Embedded Anhydrous ADP and Clopidogrel

One of the major concerns of this design is the introduction of key reagents, namely ADP and MRS 2395 (a $P2Y_{12}$ inhibitor), into the micro-channel. There are two options that achieve this goal: 1) ADP and MRS 2395 in solution phase are injected into the microchannel by mechanical penetration of the chip; or 2) They are deposited on the channel floor as dry reagents readily dissolvable by the blood stream. However, it is almost apparent that the former requires additional mechanical technology on the box design that not only necessitates micro-scale precision to carry out fluid injection but also undoubtedly increases the cost of our project. Furthermore, this mechanical addition adds extra burden on box-side cleaning. From the point of care perspective, this looks appallingly unattractive. The second option is a relatively simple alternative that solves the aforementioned problems. The incorporation of preserved reagents into a lab-on-chip device simplifies operation and increases portability. Additionally, this design translates into a classic well-defined mass transport problem that helps the design team to further explore the application of mass transport theories.

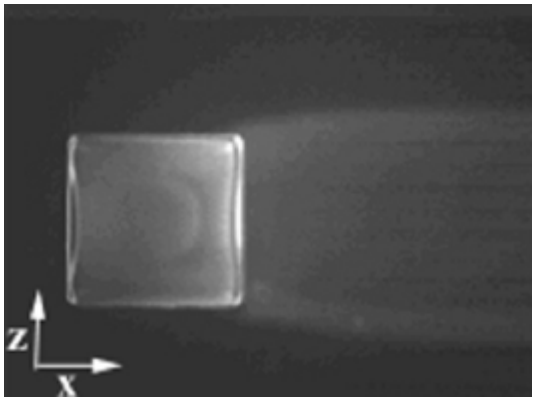


Figure 3-12: Fluorescence image of the anhydrous reagent dissolution in a flow stream (41)

Incorporation of dry reagents onto the channel often entails the addition of stabilizing preservatives. For example, some proteins are preserved by drying in tetrahalose, which stabilizes proteins by substitution of the water molecular of the hydrated proteins by the sugar

molecules, thereby maintaining the native form of the proteins. [41] Fortunately, ADP and MRS 2395 are relatively stable as dry reagents. No stabilizing reagents are required.

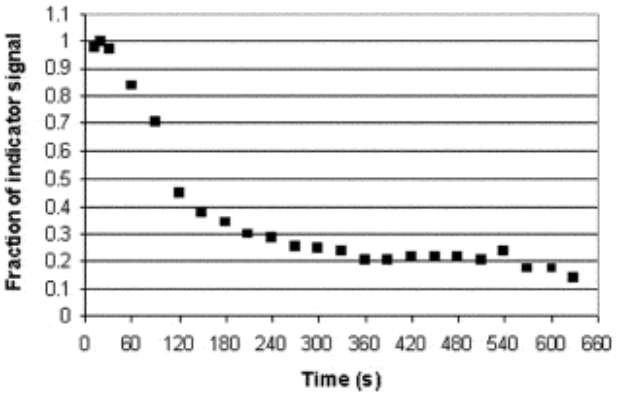


Figure 3-13: Time dependence of the dissolved reagent concentration in stream (41).

However, preserving ADP and MRS 2395 directly on the surface of the channel without some "anchoring" agents will certainly result in washout of ADP and MRS 2395 at the first few seconds of operation. By drying ADP and MRS 2395 in a heavy carbohydrate (usually Dextran), we essentially create a carbohydrate matrix that encapsulates the dry reagents. This mesh sugar structure not only helps secure the dry reagents in place but also establishes a relatively constant concentration gradient that in turn gives us spatial and temporal control of the ADP & MRS 2395 concentration in the bulk fluid.

The reagents with carbohydrate in a well-mixed solution form are deposited onto the floor of the channel by robotic pin tools. The solution is allowed to dry. Since the original solution is well-mixed, the patterning of ADP & MRS 2395 can be assumed to be uniform. The patch of dry reagents spans across the 250µm-wide channel and the length is yet to be determined.

The release of ADP and MRS 2395 from the carbohydrate matrix and their subsequent transport downstream may be controlled by parameters such as channel and patch dimensions, patch shapes, fluid flow rate, and the chemical composition of the matrices. Under the pre-specified flow rate and channel dimension, the only manipulative parameters are length of the patch and the composition of the matrix, the latter of which will not be explored further here; it

will be experimentally determined by trial and error. For the following discussion, the length of the patch is computed by invoking the *film theory* model. Our problem is can be formulated as laminar forced convection over a flat plate.

The problem can be translated into the following statement: Whole blood flows through a patch with a constant ADP concentration $C_{ADP,s}$. The dimension of the channel is specified; the width, in the y direction, is 250 µm while the height, in the z direction, is 60 µm; the edge effects

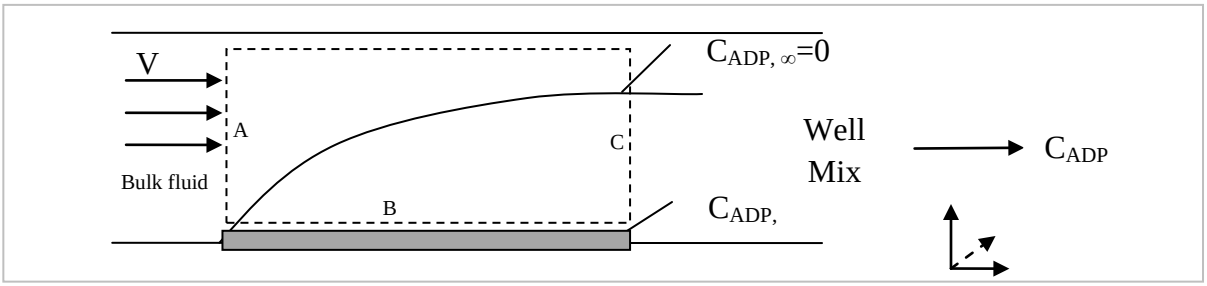


Figure 3-14: Blood flow past a ADP concentration patch

in the y direction are assumed to be negligible. A theoretical boundary is drawn in Figure 3-14 to elucidate the focus of the problem. Now, it is clear that the release of ADP from point B must equal to the amount of ADP leaving point C, which is the product of the volumetric flow rate $\dot{V}$ and the well-mixed ADP concentrationC_{ADP}. The length of the ADP strip, x, must be designed such that the flux of ADP at point B satisfies the material balance.

Under this modeling assumption, the Reynolds number, which is the ratio of inertial forces to viscous forces, is defined mathematically as the following [42]:

$$\boldsymbol{Re_x = \frac{\rho u_\infty x}{\mu} = \frac{u_\infty x}{\nu}} \qquad \textbf{(3-10)}$$

where u_∞ is the bulk fluid velocity; v is the kinematic viscosity (unit of m^2/s); and x is the position measured from the starting point of the patch. Therefore, Re_x is a not constant value across the length of the patch; it is a linear function of x.

The Schmidt number, defined as the ratio of momentum diffusivity to mass diffusivity, is used to characterize fluid flow that involves mass transfer. [42]

$$\boldsymbol{Sc = \frac{\mu}{\rho\, D_{AB}} = \frac{v}{D_{AB}}} \qquad \textbf{(3-11)}$$

in which ρ is the density of the fluid (kg/m^3); D_{AB} is diffusion coefficient (m^2/s).

The Sherwood number, which is dimensionless ratio of convective to diffusive mass transport, is defined as [42], denoted by variables with a superscript bar:

$$\boldsymbol{\overline{Sh_x} = \overline{h_m}\frac{x}{D_{AB}}} \qquad \textbf{(3-12)}$$

where h_m is the mass transfer coefficient. This definition is also valid in terms of average quantities. From now on, the bar notation denotes average quantities.

The semi-empirical mass transfer correlation corresponding to forced convection over a flat plate is taken from. [42]

$$\boldsymbol{\overline{Sh_x} = 0.664\, Re_x^{1/2}\, Sc^{1/3}} \qquad \textbf{(3-13)}$$

Equation 3-12 is substituted into Equation 3-13 and $\overline{h_m}$ is solved in terms of x:

$$\boldsymbol{\overline{h_m} = \frac{0.664\sqrt{\frac{ux}{v}}\left(\frac{\mu}{\rho D_{AB}}\right)^{1/3} D_{AB}}{x}} \qquad \textbf{(3-14)}$$

The number of ADP molecules released from the plate is proportional to the concentration gradient and the proportionality constant is $\overline{h_m} A_B$. [42]

$$\boldsymbol{n_{ADP,B} = \overline{h_m} A_B\left(C_{ADP,s} - C_{ADP,\infty}\right)} \qquad \textbf{(3-15)}$$

in which A_B or the product of the width of the channel (W) and x (Wx) is the area of the rectangular ADP patch; $C_{ADP,s}$ is the concentration of ADP at the surface, which is manipulated

experimentally; $C_{ADP,\infty}$ is the concentration of ADP initially in the bulk fluid (assumed to be 0 for now).

As discussed above, this number has be the same as the number of ADP molecules leaving at point C by material balance:

$$\boldsymbol{n_{ADP,C} = \dot{V} C_{ADP}} \quad \textbf{(3-16)}$$

$$\boldsymbol{n_{ADP,B} = n_{ADP,C}} \quad \textbf{(3-17)}$$

$$\boldsymbol{\overline{h_m}(x) A_B \left(C_{ADP,s} - C_{ADP,\infty}\right) = \dot{V} C_{ADP}} \quad \textbf{(3-18)}$$

Substituting the expression for $\overline{h_m}$ and A_B, the above equation is solved for x in terms of $C_{ADP,s}$ and we have:

$$\boldsymbol{x = \frac{2.268\, \dot{c}^2 {C_{ADP}}^2 v}{u\left(\frac{\mu}{\rho D_{AB}}\right)^{2/3} {D_{AB}}^2 {C_{ADP,s}}^2 W^2}} \quad \textbf{(3-19)}$$

Four different values of C_{ADP} are desired in our design. In increasing order, they are 0.1, 1, 10, 100 times the IC50 (which is 2mM) of ADP, or 0.2mM, 2mM, 20mM and 200mM respectively. From the above relation, it is clear that the required length of the patch is proportional to ${C_{ADP}}^2$. If $C_{ADP,s}$(the surface concentration of ADP) is set constant, x could range from 100um for 0.2mM to 100m for 200mM. This range is not physically feasible in a chip with a length of 6cm. On the other hand, x can be set constant, 150um for the sake of consistent dimensional design, and $C_{ADP,s}$ is varied to achieve the 4 specified concentration of ADP. The above relation is arranged to solve for $C_{ADP,s}$ in terms of C_{ADP}.

$$\boldsymbol{C_{ADP,s} = \left(\frac{1.506\dot{V}}{\sqrt{\frac{ux}{v}}\left(\frac{\mu}{\rho D_{AB}}\right)^{\frac{1}{3}} D_{AB} W}\right) C_{ADP}} \quad \textbf{(3-20)}$$

Previously, we make the assumption that $C_{ADP,\infty}$ is approxomaely 0. To validate this claim, we have to ensure that the boundary layer near the end of the patch does exceed the channel height of 60 μm [42]:

$$\boldsymbol{\delta_c \approx \frac{5x}{Sc^{1/3}\sqrt{Re_x}} < 60\ \mu m} \qquad \textbf{(3-21)}$$

Detailed calculation is performed in the Appendix 7.4. The results are tabulated:

$C_{ADP,s}$ (uM)	IC_{50}	$C_{MRS2395}$ (uM)	x (um)	Re_x	Sc	Sh	δ_C (um)
0	0x	0	149	2.18	705.5	8.7	57
24.8	0.1x	0.36	149	2.18	705.5	8.7	57
248	1x	3.6	149	2.18	705.5	8.7	57
2480	10x	36	149	2.18	705.5	8.7	57

Table 3-3: The required surface ADP concentration corresponding to in-stream ADP concentration (in red)

In conclusion, these values are only approximations. For further development of our design, experiments are necessary to extract better estimates of these quantities. However, the numbers and equations here serve as initial guesses for future trial and error experiments and provide insight into the relationship between critical to quality variables.

3.2.5.1 Laminar Forced Convection over a Flat Plate

Peclet number, defined as the dimensionless ratio of convective motion to diffusive motion, is another insightful indictor of fluid mixing behavior. On micro-scale, this diffusive solute motion is slow relative to the convection of the solution along the channel, or Pe is large. With our channel dimension specification, the Peclet number is computed as the following:

$$Pe = \frac{ul}{D} = \frac{(1\times10^{-2}m/s)(9.68\times10^{-5}m)}{10^{-10}m^2/s} = 9680 \gg 100 \quad \text{(3-22)}$$

where *D* is the molecular diffusivity, u is the velocity, l is the effective diameter of the rectangular channel.

The small Re, implying the absence of turbulence, and the large Pe, meaning slow diffusive solute motion, suggest the length required for mixing is unreasonably long. For such unperturbed flows, the approximate distance along the channel required for mixing to occur is suggested by [43]

$$\Delta y_m \approx u \times \left(\frac{l^2}{D}\right) = Pe \times l = 9680 \times 9.68 \times 10^{-5}m = 0.93m \quad \text{(3-23)}$$

This length is reasonably long given the dimensional limitation of the chip. Adding to its disadvantage, the unperturbed flow requires mixing length that grows linearly with velocity/Pe, rendering the re-usability of the pre-designed micro-channel at different flow conditions impossible.

To circumvent this problem, we need to introduce a structure that mimics the effect of turbulence. There are 3 options that have been intensely and thoroughly researched: squarewave, serpentine and herringbone. Due to the simplicity of its fabrication, the herringbone configuration is generally deemed to be one of the most cost-effective designs for mixing in micro-channels. With PDMS as the raw material for chip fabrication, the mold for the herringbone grooves were made possible by a two-level photo-lithography. These grooves which were recessed into the floor of the flowing channel induce transverse components of flow that stretch and fold volumes of fluid. These stirring flow

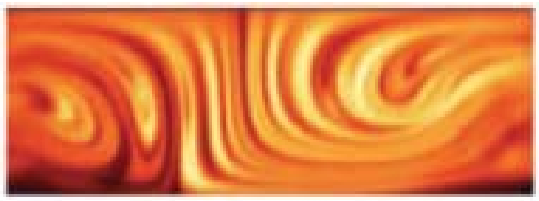


Figure 3-15: Cross-sectional view of fluid flow pattern in the micro-channel (43)

patterns reduce the average distance over which diffusion must act in the transverse direction to homogenize unmixed volumes. (43) As a result, mixing length is substantially reduced. Before we continue, it is deemed necessary to briefly review the concept of mixing in micro-channels. The quality of mixing can be characterized by coefficient of variation (CV). Mixing analysis generally assumes there are two steams flowing side by side; one contains the solutes dissolved uniformly in solvent and the other is pure solvent. The CV quantifies the degree of concentration variation between the two streams. Experimentally, the degree of mixing is evaluated after confocal microscopy is performed on the mixing streams. The CV can be extrapolated from image analytical techniques. Since the initial design of the herringbone mixer, analytical modeling of it becomes an intense area of research. The most complex methods involve computational fluid dynamics (CFD) modeling, which considers the redistribution of streamlines and reduction of striation thickness. Though these methods generally give a comprehensive picture of the mixer, the mathematical modeling can be prohibitively challenging, if not impossible.

A simple analytical model accompanied the herringbone mixer adapted from [44] with minimum modification due to similarities in design specification is presented here to analyze the degree of mixing as a function of Pe and length by assuming uniform residence time distribution of SHM. At molecular level, solute in each fluid element must diffuse to the other fluid for mixing to occur. Diffusion from a point source follows a normal distribution, and the mean magnitude is denoted by $L_d = \sqrt{2}Dt$, where

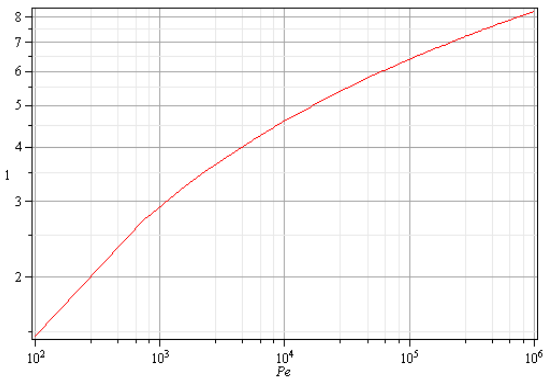


Figure 3-16: Exact solution of I_{01} vs. Pe in semi-log plot

D is diffusivity and t is the duration of the diffusion. The probability that the solute has diffused the distance L_d with the boundary limit of L_i is computed by [44] as:

$$\boldsymbol{P\left(L_d < L_i\right) = \frac{1}{2} erf\left(\frac{2\sqrt{2}L_i}{L_d}\right) \approx \frac{1.60L_i}{L_d}} \quad \textbf{(3-24)}$$

The expression for L_d is substituted with the definition of Pe. The following expression form [44] is obtained:

$$\boldsymbol{P\left(L_d < L_i\right) = 1.60\sqrt{\frac{Pe}{wld}}L_i} \quad \textbf{(3-25)}$$

The empirical relation between l and L_i is computed by MATLAB with COMSOL data by [44]:

$$\boldsymbol{L_i = 19.7\exp(-0.55\, l) \qquad R^2 = 0.994} \quad \textbf{(3-26)}$$

This is substituted into the previous equation and solved for $l_{0.1}$ with an arbitrary cutoff probability of 0.1. The exact solution is generated by Maple 11 and graphed (Figure 3-16). This is approximation of the solution

$$\boldsymbol{l_{0.1} \approx 1.73\log(Pe) - 2.16} \quad \textbf{(3-27)}$$

The exact value of $l_{0.1}$is 4.58 cycles corresponding to Pe of 9680 from Figure 3-16. This value computed by using the analytical model agrees to a reasonable extent with the experimental value (Figure 3-17). It is important to note that l is expressed in terms of cycle numbers; therefore the length per cycle multiplies the cycle number, l, to give the actual length of the mixer.

$$\boldsymbol{L = l_{0.1} \times d = 4.58d} \quad \textbf{(3-28)}$$

where d is the length of one cycle, which is computed as the following:

$$\boldsymbol{d = distance\ between\ adjacent\ grooves\ measured\ from\ the\ center \times the\ numer\ of\ grooves\ per\ cycle = 145um \times 16 = 0.232\ cm} \quad \textbf{(3-29)}$$

The length can then be computed to be 1.06 cm. Most importantly, note that the mixing length, l is proportional to log(Pe), implying more "resistance" to perturbations in flow rate. This elegant feature introduces stability and re-usability in our chip design, as this configuration can be employed to give only slight changes in mixing length under different flow conditions. In addition, the mixing length is reduced from 93 cm to about 1cm, a scale that is compatible with our chip dimension. Although 1 cm is manageable in terms of size, additional manipulative design is required to efficiently maximum the space utilization.

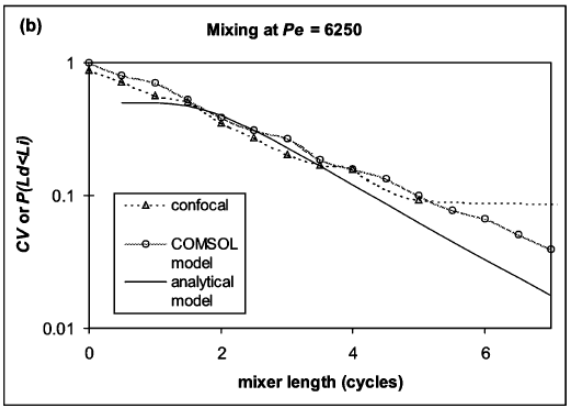


Figure 3-17: Comparison of analytical model and experimental data (44)

Due to the chip dimensional constraint (with a width of 4 cm and a length of 6 cm), the herringbone supplemented micro-channel mixer is still relatively long and should not be linearized; it must be in a compacted form to accommodate the rest of the components of the chip. To achieve this purpose, the squarewave configuration is employed.

3.3 MCCRA Device

The basic concept of the device is a box type structure into which the disposable chip can be inserted. Once inserted, a vacuum will puncture through the PDMS into the waste reservoir in

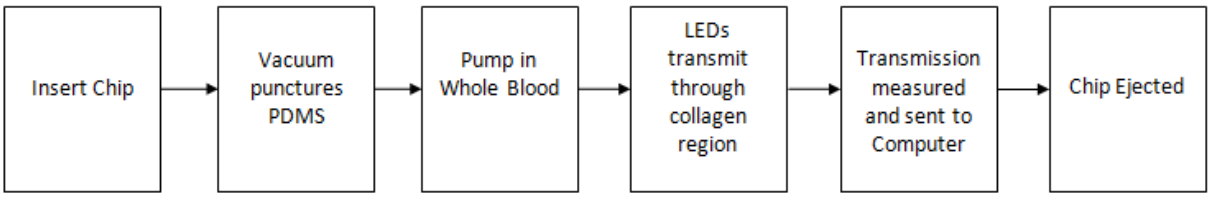


Figure 3-18 Flow chart showing the basic mechanisms of the device's operation.

order to provide the negative pressure required to make the blood flow through the chip. Also within the device will be a row of eight white light emitting diodes (LEDs) situated above where the collagen portion of the chip will sit. Below the lights there will be a transmission detector. Readings from the detector will be transmitted to an electronic device that will be connected to a computer. The device will convert the readings into the necessary format to be read by the computer program. (This analysis and graphing software will be developed by a hired programmer). The program will display a comparison of the transmission data to the IC50 curve for MRS 2395. Accompanying the program will be a manual of standard ranges for healthy individuals and the clinical implications of variations from these ranges, based on data acquired through testing and clinical trials. From this the doctor can interpret the results and determine the proper clopidogrel dosing amount. The vacuum will run for a total of 5 minutes, and readings will be taken in 5 second intervals. Once the data has been collected, the chip will be ejected from the device (See Figure 3-18).

Due to limited knowledge of electronics and electrical engineering, a consultant advised the outsourcing of the manufacturing of the device. The consultant suggested a “black box” style device, into which the MCCRA’s specifications can be added and an already existing electronic “reader” on the box can be programmed to this product’s specific needs.

3.3.1 Instruction for Device Usage

Press “on” button to turn on device. Remove MCCRA chip from refrigerator, and then remove from the sterile packaging. Put patient’s blood sample onto designated location on the MCCRA chip. Insert chip into device, and press “start” button. When device has finished operation, the red “complete” light will turn on. Remove chip from device and dispose in

hazardous/biological waste container. Use computer readout and compare to MRS 2395 IC50 curves to determine dosing amount.

3.4 Data Analysis

3.4.1 Prototype Findings

The photomask was designed in LayoutEditor[3]. In order to probe the feasibility of the design of our chip, a high-resolution (10,000 dpi) transparency of the chip design was printed[4] KMPR 1050 positive photoresist[5] was spun on a silicon wafer[6] on a CEE 100[7] spinner for 30 seconds at 3000 rpm to achieve a thickness of 62μm[8]. Following a soft bake at 100C for 15 minutes, the wafer was aligned with the aforementioned mask using a Karl Suss MA4[9] mask aligner, then exposed to 365nm light at a constant intensity of 5mW/cm^2 for 195 seconds to manufacturer's recommendations of 975mJ/cm^2 exposure energy. Following a post exposure bake at 100C for 3 minutes, the unreacted photoresist was removed with AZ 300 MIF[10] developer for 10 minutes under constant agitation. After fabrication, the master was silaized for three hours with 1,1,2,2-

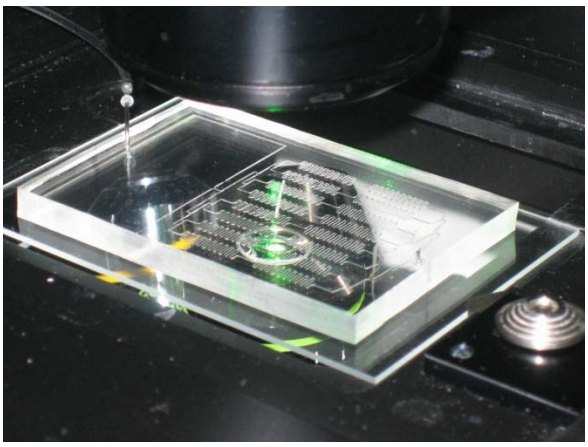


Figure 3-19 PDMS block consisting of our chip design. Presence of defined and correctly patterned features, such as channels, mixing regions and reservoirs, has instilled confidence in our product design. Note: the upper left section of the chip shows the vacuum being punctured into the waste well.

[3] Jürgen Thies
[4] CAD/Art Services, Bandon, OR
[5] Microchem, Newton, MA
[6] 100mm diameter, <100>, Virginia Semiconductor, Fredericksburg, VA
[7] Brewer Science Inc., Rolla MA
[8] measured post-fabrication using a Tencor Alpha Step (KLA-Tencor Corp, San Jose, CA)
[9] Karl suss, Garching, Germany
[10] Clariant Corp., Somerville, NJ

tetrahydrooctyle-1-trichlorosilane[11] to facilitate PDMS removal. The master was used to fabricate the mold in a process similar to that of the manufacturing mold design. 80mL of PDMS pre-polymer was mixed at a 1:10 ratio with curing agent[12] and degassed, then poured onto the master. This device was placed in an 80°C oven for two hours, and then removed, cut from the mold, and ports punched. (45)

For proof of concept purposes, a number of analogous yet simplified chip designs were included on the master in addition to the intended microfluidic device. The simplified analogue consisted of only one of the eight channels. The same vacuum setup that would have been used for the more complex microfluidic chip was used for the single channel. The prototype testing was therefore intended to prove three things: that the chip design was capable of being manufactured, that MRS 2395 inhibits aggregation of platelets, and that platelet aggregation would occur on the collagen. Positive evidence for these factors would indicate that hard

Figure 3-21 A Harvard PHD 2000 Syringe Pump was used as the driving force for flow in our prototype.
The syringe pump was set at a speed that mimicked the sheer rate of blood found *in vivo*.

Figure 3-20 Microscope setup used to record platelet aggregation by measuring changes in light transmittance.

[11] United Chemical Technologies, Bristol, PA
[12] Sylgard 184, Dow Corning, Midland, MI

to measure system characteristics, such as specific concentration gradients, were correctly calculated.

With the microfluidic chip fabricated, the design was now ready to be tested. However, lab equipment needed to be used as substitutes for functions the box would normally perform. A lab-scale syringe pump[13] was substituted for the vacuum pump to apply a constant flow rate across the channel. Upon obtaining blood from a willing volunteer, it was allowed to mix off chip with ADP in a 1.5 mL tube. From this mixture, approximately 200 μL were applied to one end of the channel while the syringe pump was attached to the other end of the channel. Similar to the way our box will assay the chip, the prototype was mounted on an inverted microscope[14] and light transmittance through the collagen patch was measured as a function of time. Images were collected every five seconds over a five minute period. To get an accurate numerical image of the light transmittance, a 10X objective was used. After a few trials, the experiment clearly showed the aggregation of platelets on the surface of the collagen. To bridge the gap between prototype and desired product even further, the blood and ADP mixture was incubated with varying concentrations of MRS 2395 and platelet aggregation was further tested.

3.4.2 Image J

Although the MCCRA will utilize a sophisticated computer algorithm capable of advanced interpretation and manipulation of recorded data and error detection ability, the prototype findings were susceptible to analysis with the ImageJ Image Processing program.

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[13] PHD 2000, Harvard Apparatus, Holliston, MA

[14] Olympus IX81, Olympus America, Center Valley, PA

As the blood was run through the chip, a CCD camera[15] captured a black and white image of the channel every 5 seconds over the experimental time period of five minutes. These pictures were then uploaded to ImageJ, where a region of interest was selected around the collagen patch of the channel and another area was drawn around an upstream part of the channel devoid of collagen. The average grayness of the boxes (dark boxes equals less surface height, lighter surface equals higher surface height) were determined from intensity profiles from those regions, and the difference between the average pixel intensity of the collagen-free area and collagen area was calculated. This value was divided by the average intensity of the collagen-free region and relative intensity value was determined. These values were plotted versus time and the trends were observed.

3.4.3 Results

As found in Figure 3-22, blood plus ADP initially mixed with inhibitor at 5, 10 and 50 μM was flowed over a collagen patch. The y-axis plots the relative grayness of the patch, which is a measure of the topology of the surface. The graphs all individually show a transient regime where platelet aggregation begins and increases steadily. A steady state value for the signal is then attained, normally when the experimental time equaled about 250 seconds. Compared against each other, the experimental trials lead to the conclusion that a more concentrated MRS 2395 solution leads to less platelet aggregation. In addition, the experiments using 10 μM and 50 μM inhibitor show graphs that lead to a similar steady state platelet aggregation. These graphs imply that a boundary is reached at above 10 μM MRS 2395, and thus any additional inhibitor is without effect.

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[15] ORCA-ER, Hamamatsu, Bridgewater, NJ

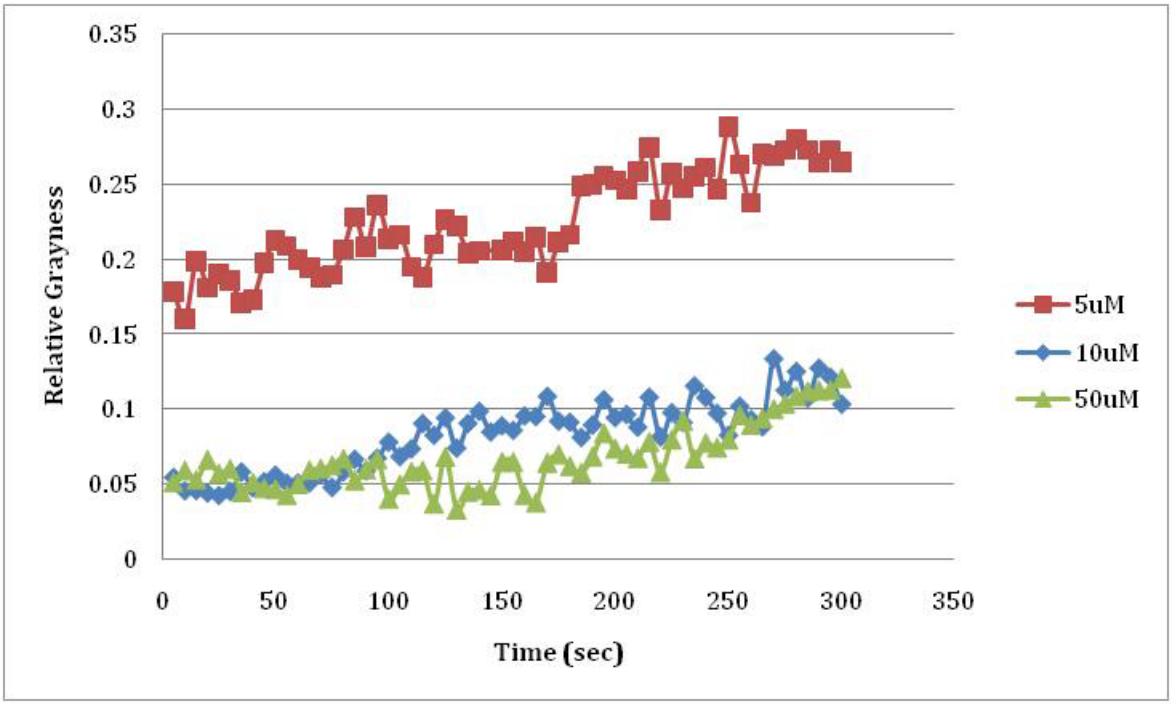

Figure 3-22 The platelet aggregation is recorded as a function of time.
Aggregation was determined from a measure of pixel grayness, which is an indication of the surface topology. A darker surface (lower pixel grayness value) translated to less surface structures, while a lighter surface (higher pixel grayness value) meant higher surface structures.

3.4.4 Dose Response Curve

The steady state values from the experiments were used to construct a very elementary dose-response curve. Shown in Figure 3-23, the graph demonstrates the effects of the increasing MRS 2395 concentration on platelet aggregation. Does-response curves are normally characterized by a sigmoidal shape, where the steepest (slope) portion of the curve is located in the middle of the graph, while the lower and higher

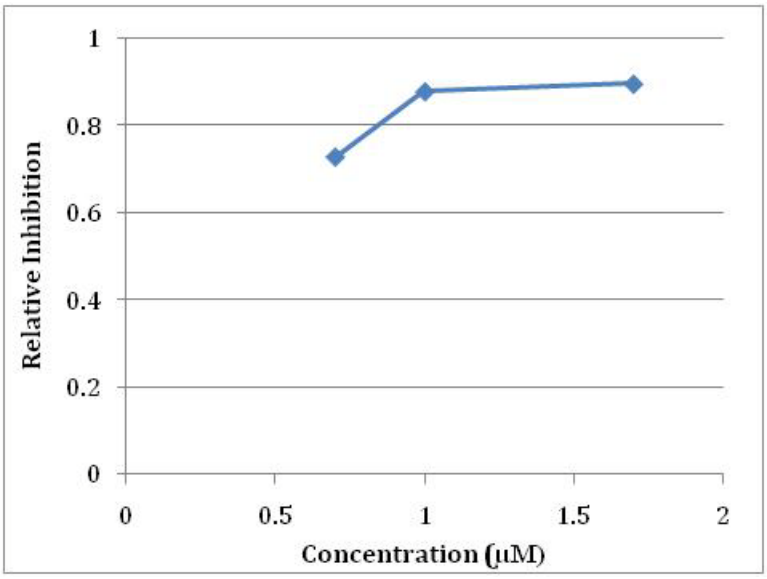

Figure 3-23 The results of increasing inhibitor concentration on platelet aggregation. The points shown represent the upper portion of the sigmoidal dose-response curve.

points show little change. Expecting the results in Figure 3-23 to be similar, the data points collected must occur after the inflection point of the sigmoidal curve. Therefore, the concentration of inhibitor used in this study must have been above the concentration that gives half inhibition of receptors and gives us an idea of the IC_{50} for MRS 2395 against the $P2Y_{12}$ receptor. One study of rat platelet aggregation found an IC_{50} of 3.6 μM, corresponding well with the data found here. [46]

The curve serves to successfully prove the contention that MRS 2395 functions to inhibit platelet aggregation. Since it behaves as intended, the inhibitor should be successful in allowing the MCCRA to ascertain the amount of $P2Y_{12}$ receptors that have not been inhibited clopidogrel. Thus, all of the proof of concept points have been accurately answered and the prototype validated.

3.5 Financial Analysis

3.5.1 Market Share and Sales Project

It has been shown that pre-treatment with clopidogrel before primary percutaneous coronary intervention (PCI) lowers the risk of future complications based angiographic outcomes, thus significantly reducing the risk of cardiovascular death. [47] [48] The number of PCI cases performed annually is 1.1 million in the US as of 2008. Since the benefit from pre-treatment with clopidogrel before PCI has been known for at least 2 years, 90% of the patients requiring PCI, or about 1million, are currently pre-treated with clopidogrel. Presumably, all clopidogrel prescription requires dosage measurements.

For our project development, one year (Year 2010) is allocated for product design and development from the prototype. The Food and Drug Administration approval and regulation

processes are generally expected for startup biotech devices that are used in health care settings. This process is anticipated to take 2 years (Year 2011 and 2012), a relatively short period due to the non-invasive nature of our device. The expected date for launching our product is the beginning of 2013 and it is expected to initially capture 10% of the market share (1 million) or 100,000 patients. It is anticipated this number will grow thereafter due to the increasing awareness of the benefits from clopidogrel and the competitive advantage of our product. The market share is expected to follow a step increase from 10% initially to 30% for the second year of operation, and finally 50% until the end of operation.

Previously it has been shown that the market for clopidogrel resistance detection is fiercely competitive (See Competitive Analysis 2.8). Several devices employing different clotting quantification techniques (for example, florescence transmittance aggregometry, turbidity measurement, electro-conductivity measurement etc) are competing to be the gold standard. Their prices per test range from $50 to $100, while their run times plus incubation vary from 13 minutes to hours. If the time is considered an opportunity cost, the most cost-effective price and run-time combination charges 650 min*dollar/test (VerifyNow). As the world is looking for a cost-effective, high-throughput device that efficiently measures clopidogrel resistance with sophisticated error checking mechanisms, we introduce our POC high-throughput screening device that utilizes high performance microfluidic technology that enables us to charge only $100 per 4 tests per 10 minutes (250 min*dollar/test). From the time and cost perspectives, our product is expected to be at least 2.5 times more efficient than any of its existing competitors.

In the subsequent revenue estimation, monetary figures are expressed in real terms (in today's money value). Inflation is not incorporated into our financial models, because it is

impracticable to accurately predict inflation rate based on historical data. Furthermore, it is important to note that cash flow discounting (CFD) using quantities in real terms with the real discount rate is theoretically equivalent to CFD using quantities in nominal terms with nominal discount rate. It is clear that estimating inflation rate and adjusting discounted revenues and costs accordingly is superfluous. The real discount rate is related to the nominal discount rate and inflation rate by the Fisher equation:

$$\frac{1+Nominal\ Discount\ Rate}{1+Inflation\ Rate} - 1 = Real\ Discount\ Rate \qquad (3\text{-}30)$$

Cash Flow Discount (CFD) will be discussed in more detail in later sections. Our plant operation is anticipated to last 7 years, after which the patent expires or our product loses its competitive edge to new technologies. The annual revenue from MCCRA chip sales is summarized in the following table:

Year	2013	2014	2015	2016-2019
% Market	10%	30%	50%	50%
Test/Yr	100,000	300,000	500,000	500,000
Chip Price	$100	$100	$100	$100
Revenue	$10,000,000	$30,000,000	$50,000,000	$50,000,000

Table 3-4 Revenue Table

3.5.2 Expenses

Our design group has attempted to get in contact with sales representatives from various chemical suppliers. A rough estimate of the costs of the raw materials for the chips, equipments, and general expenses were obtained. Due diligence in the form of thorough research was performed to verify the quotes from these sales representatives. It was recognized that these prices might represent an overestimation because quantities would be purchased in bulk, resulting in a discount. Moreover, if the project was carried beyond the design stage, better deals

with the suppliers might be negotiated. For the purpose of initial design, the prices found were satisfactory. For subsequent cost analyses, the expense worksheet is dissected into the following 3 categories: 1) total initial capital investment 2) variable costs, 3) fixed costs. Each of these will be discussed in detail in the following subsections.

3.5.2.1 Total Capital Investment

Table 3-5 provides a summary of the items that are part of property, plant and equipment (PPE). The focus of our project is product design, so all will be pre-fabricated upon purchase from suppliers. However, before the purchase of some of these pieces of equipment (such as the oven, stirred tank, and cold room storage), equipment sizing has been performed (Appendix 7.7). With these sizing estimations, equipment with relevant capacities is chosen to avoid needless costs. For example, the necessary storage space to accommodate a monthly inventory of 42,000 chips is computed to be 70 cubic feet, while the refrigerator unit has a volume capacity of 78 cubic feet. The sizing-aided selections of other equipments are carried out in a similar fashion.

As mentioned previously, the quotes for all equipments are obtained from sales representative and supplier websites. For example, Silverson Laboratory Scale Batch Mixer costs about $1,500, taken from the Silverson website. Bare modules factor for this item is 1.0 because there is no extra installation fee associated with it; the mixer is pre-fabricated and pre-configured upon purchase. The bare module cost (C_{BM}) is the product of the bare module factor (F_{BM}) and the purchase cost (C_P):

$$\boldsymbol{C_{BM} = F_{BM} \times C_P} \qquad \textbf{(3-31)}$$

The bare module costs for all other process equipments, the storage, security system, and lab computers & software are computed in a similar fashion.

Equipments and Related Costs

Process Machinery		Purchase Cost	Bare Module Factor	Bare Module Cost	Purchase Year
	Wisconsin Industrial Oven	$ 20,000	2	$ 40,000	2010
	Biomek FX Robotic Arm	$ 15,000	1.5	$ 22,500	2010
	Tankmaster UV Sterilizer	$ 1,500	1.5	$ 2,250	2010
	Silverson Batch Mixer	$ 1,500	1	$ 1,500	2010
	Basic Mounting Plate for Beckman FX Gripper	$ 400	1.5	$ 600	2010
	384 Pin Tool Head	$ 7,600	1.5	$ 11,400	2010
Storage		Purchase Cost	Bare Module Factor	Bare Module Cost	Purchase Year
	McQueen Labs Refrigerator 78cf	$ 8,900	1	$ 8,900	2010
Other Equipment		Purchase Cost	Bare Module Factor	Bare Module Cost	Purchase Year
	Security System			$ 2,000	2010
	Approval Yr 1			$ 2,500,000	2011
	Approval Yr 2			$ 2,500,000	2012
	Box (free trial)			$ 273,500	2013
Total Bare Module Costs:	**$5,362,700**				

Cost of Site Preparations:	**5.0% of Total Bare Module Costs = $3,300**
Cost of Service Facilities:	**5.0% of Total Bare Module Costs = $3,300**
Direct Permanent Investment:	**Sum of the above = $5,345,200**
Cost of Contingencies and Contractor Fees:	**18.0% of Direct Permanent Investment = $12900**
Cost of Plant Start-Up:	**10.0% of Total Depreciable Capital = $8,500**
Working Capital:	**12% of sales = $6,000,000**
Total Capital Investment:	**Sum of all = $11,366,500**

Table 3-5 Equipment and Related Costs

It should be noted that bare module factors associated with different equipments are not the same. For example, the bare module factor of 1.5 for Biomek FX Robotic Arm reflects that the cost of installation is 50% of the purchase cost, while a bare module factor of 2 for Wisconsin Industrial Oven suggests that another 100% of the purchase cost is used in setting ventilation system associated with its installation. All of the aforementioned items are purchased during the design stage in 2010. The rest of the section is devoted to addressing the box and FDA approval expenses.

The box manufacture will be out-sourced to an electronic supplier. A list of functional specifications will be provided to the box manufacturer to customize the box. Table 3-6 summarizes the components in the box as well the cost of assembly (which is estimated to be 50% of the aggregate purchase costs of individual components). It is essential to note that the box will not be processed by our chip manufacturing plant and one box is directly distributed to one hospital that hold contract with our service. The box selling price is the same as the selling price required by the electronic supplier; in another words, we make no profit out of the sales of the box. This should be deemed reasonable because we as the chip manufacturing side do not add extra value to the box. Therefore, the box sales will not appear in our financial analysis, while box maintenance and technical support will be considered. In Table 3-5, there is a "Box free trial" entry under "Other Equipments". One major reason is that most of the hospitals (we are targeting about 4000 large hospitals in the US) already purchased our competitors' boxes; these expenses represent sunk costs to them, costs that they incurred in the past. For this reason, they might not be willing to dispose these boxes and replace them with ours. Hence, we will purchase 50 boxes and offer them as free trial products to 50 selected hospitals in 2013 (1^{st} year of

operation). This business strategy will not only ensure us market share, but also support our marketing effort.

The cost of FDA approval excluding overhead expenditure is assumed to be 10% of the annual sales at full capacity, or $5 MM, evenly distributed over the 2 year period (2011 and 2012). Overhead is defined as all costs but direct labor and materials on the income statement. This is treated as "other equipment" in the profitability worksheet. It is important to recognize FDA approval is not a one-time cost. Congress enacted the Medical Device Amendments of 1976 to further FDA regulation on the safety and effectiveness of medical devices [(49)]. The medical device regulation generally can be divided into 2 stages: 1) pre-market evaluation and approval process and 2) post-market evaluation. This implies overhead expenditures will be allocated to deal with federal regulation on an annual basis. Therefore, for economic analysis at the design stage, it is convenient and reasonable to treat the $5 MM spent on FDA approval as depreciable capital investment, and overhead is computed as a percentage of this amount annually. This will be discussed in detail in the section on (Fixed Costs 3.5.2.3). Some of the plant equipments and process facilities need modifications of the factory for installation. For example, ventilation system might have to be set up before installing the oven. We account for this type of expenses within Cost of Site Preparation in Table 3-5, which is taken as 5% of bare module costs. [(50)] To maintain a well-managed, orderly factory, service facilities are indispensable and take about 5% of bare module costs. [(50)] The sum of bare module costs, cost of site preparation and service facilities is denoted as the Direct Permanent Investment (DPI). The cost of contingencies and contractor fees is about 18% of DPI. The accumulated sum of DPI and cost of contingencies and contractor fees is known as Total Depreciable Capital (TDC). The cost of land is not included in the section because the manufacturing facility will be rented. The

annual rent payment is treated as fixed cost in annual basis in the section on Fixed Costs. Finally, there are costs incurred for the plant start-up, which is usually 2% of TDC [50]. Working capital, which includes accounts receivable, cash reserves, accounts payable and inventory, is about 12% of annual sales at full capacity, or 1 month of sales revenue. The sum of the aforementioned items is lumped together as the Total Capital Investment (TCI). It should be noted that TCI is a one-time cost, in contrast with fixed costs and variable costs, which we incur on an annual basis.

Box Components	
PC	
Hardware	$500
Software	$5
Component & Instrumentation Selection	
Power Supply	$50
Vacuum	$100
LED	$200
Detector	$25
Interlock Switch	$25
Box	$100
Wire	$200
Mechanical Parts	
Box Mods	$250
Chip Hoder Brackets	$200
Chip Vacuum Cover	$250
LED Bracket	$200
Detector Bracket	$200
Box Lid with Interlock	$250
Silk Screens/Labeling	$250
Sum	$2,805
Bare Module Factor	1.50
Bare Module Cost	4207.5
Required Return	0.3
Box Cost	**5470**

Table 3-6 The box price is estimated by taking the sum of the costs of individual components and adding the installation cost and required profit from the out sourced company.

3.5.2.2 Variable Cost

The variable cost is defined as the cost per unit of merchandise produced. It is partitioned into 3 major categories: raw materials, utilities, general expenses. As mentioned previously, the quotes for the raw materials expressed in terms of either per volume basis or per mass basis are obtained from supplier sales representatives. Material balance is carried out to compute the amount of each reagent needed to produce 1 chip (refer to section on chip manufacture). With this, the cost of each reagent per chip can be calculated (Table 3-7).

In the United States, energy consumption is reported ranging from $1.50 to $2.50 per square foot in a typical building [51]. With area occupancy of 2500 square feet, our plant utility cost can reach up to $6250.

This is considered the most basic cost in our manufacturing plant. However, the most utility-consuming equipments that are used in chip production include a group comprised of the oven, robotic pin tools and the packaging machine. The power of all these equipment can be found in their product descriptions and multiplied by operation time and cost per utility unit to give the costs of utilities expended by them. The overall cost of utility is the sum of the cost for a typical building and the cost from utility heavy equipments. Nonetheless, for simplicity, we just multiple a factor of 1.2 to account for extra cost of utilities by these equipments here:

$$\boldsymbol{Utility\ Cost = 1.2 \times Typical\ Cost\ for\ a\ building = 1.2 \times 6250 = \$7500\ or\ \$0.015/Chip} \quad \textbf{(3-32)}$$

The cost items listed above are the most obvious or direct expenses incurred in producing chips. However, the less obvious or indirect costs are lumped together as General Expenses, which include transportation, direct research, allocated research, administrative expense and management incentives. All these expense items are computed as percentages

of annual sales: 3% for product transfer, 4.8% for direct research, 0.5% for allocated research, 2% for administrative expense and 1.25% for management incentive compensation (or bonus). These are normally considered variable costs instead of fixed costs because annual sales actually vary for the first 3 years of operation.

Variable Cost Summary

April, 2009

MCCRA

		Per Unit Chip		TOTAL
Raw Materials				
	MRS2395	$4.3180 per Unit of Chip	$2,159,000	
	PDMS/Curing Agent	$0.2400 per Unit of Chip	$120,000	
	Acetone	$0.5600 per Unit of Chip	$280,000	
	Collagen	$7.0000E-05 per Unit of Chip	$35	
	ADP	$0.0164 per Unit of Chip	$8,200	
	Glass Slide	$0.6250 per Unit of Chip	$312,500	
	Hydrophobic Coated Pins (20nL)	$0.0800 per Unit of Chip	$40,000	
	Hydrophobic Coated Pins (10nL)	$0.0900 per Unit of Chip	$45,000	
	Hydrophobic Coated Pin (4nL)	$0.0900 per Unit of Chip	$45,000	
	Total Raw Materials:	**$6.0195 per Unit of Chip**	$3,009,700	**$3,009,700**
Utilties				
	Electricity	$0.0150 per Unit of Chip	$7,500	
	Total Utilities:	**$0.0150 per Unit of Chip**	$7,500	**$3,017,200**
Byproducts				
	Total Byproducts:	**$0 per Unit of Chip**	$0	**$3,017,200**
General Expenses				
	Selling / Transfer:	$3.0000 per Unit of Chip	$1,500,000	
	Direct Research:	$4.8000 per Unit of Chip	$2,400,000	
	Allocated Research:	$0.5000 per Unit of Chip	$250,000	
	Administrative Expense:	$2.0000 per Unit of Chip	$1,000,000	
	Management Incentives:	$1.2500 per Unit of Chip	$625,000	
	Total General Expenses:	**$11.55 per Unit of Chip**	$5,775,000	**$8,792,200**
TOTAL		**$17.5845 per Unit of Chip**	**$8,792,200**	**$8,792,200**

Table 3-7 Costs of Each Reagent per Chip

3.5.2.3 Fixed Cost

The fixed cost includes the costs from annual operation, maintenance, operating overhead, property taxes and insurance, and space rental. In the rest of the section, a comprehensive review of each cost is given.

Operations include 1) direct wages and benefits (DWB), 2) direct salaries and benefits (DSB) and 3) operating supplies and services (OSS). To compute DWB, it is deemed necessary to elucidate a few operating conditions. Firstly, the operating hour is allotted 10 hr per day such that the manufacturing process can achieve the production of 4000 chips daily. Secondly, the number of operation days per year is assumed to be 250, or 70% of 360 days/1 year. The other 30% of the time is spent on maintenance and inspection; this is a typical assumption for a biomedical manufacturing plant. [50] Thirdly, the number of operators per shift is estimated by considering the amount labor required for each process in the production chain (Table 3-8). Lastly, the hourly rate of $42.5/hr is adopted from a similar project titled "High throughput screening of kinase inhibitors" in [50] direct wages and benefits are the following function of the aforementioned variables:

Type of Process	Number of Operators per Process Section
Mixing of PDMS and curing reagents	1
Pouring PDMS onto molds	2
Curing in oven	1
Depositing ADP, MRS, and collagen	5
Sealing chip with glass slide	3
Packaging	3
Total	15

Table 3-8 Estimation of the number of operators.

$$\boldsymbol{Direct\ Wages\&Benefits} = \left(\mathbf{15}\frac{\boldsymbol{Operators}}{\boldsymbol{shift}}\right)(\mathbf{1}\ \boldsymbol{shift})\left(\mathbf{2500}\ \frac{\boldsymbol{hr}}{\boldsymbol{yr}} - \boldsymbol{operation}\right)(\frac{\$\mathbf{42.5}}{\boldsymbol{hr}}) \qquad \textbf{(3-33)}$$

To continue our calculation of direct salaries and benefits (DSB), it is necessary to clarify the distinction between DSB and DWB. Direct wages and benefits are paid to operators who are directly involved in production while direct salaries and benefits are compensated to supervisory and engineering personnel who oversees the operation. Direct salaries and benefits is usually 15% of DWB and added to DWB to give the total labor cost of plant operation (50). It is clear labor itself will not accomplish the entire operation. Operating supplies and services are generally essential and usually taken as 6% of DWB [(50)]; these are the tools beside the plant equipments that the operators use to carry out their tasks.

Similarly, maintenance cost include 1) Maintenance Wages and Benefits (MWB), 2) Maintenance Salaries and Benefits (MSB), 3) Materials and Services (analogous to operating supplies and services), and 4) Maintenance Overhead. The distinction between MWB and MSB is analogous to the difference between DWB and DSB; generally salaries refer to supervisory personnel compensation in this report. Since maintenance is done on the plant equipment (Total Depreciable Capital), MWB should be proportional to TDC; in fact, MWB is taken as 4.5% of TDC here. It is common practice to take 25% of MWB as Maintenance Salaries and Benefits. Materials and services take into account for spare parts and external technical support for repairs. This is usually 100% of the Maintenance Wages and Benefits because machine parts and repair services are quite hefty. Accounting fees, legal fees, utilities etc. that we incur during maintenance are classified as maintenance overhead, which is 5% of MWB, typical for a biomedical manufacturing plant. [(50)]

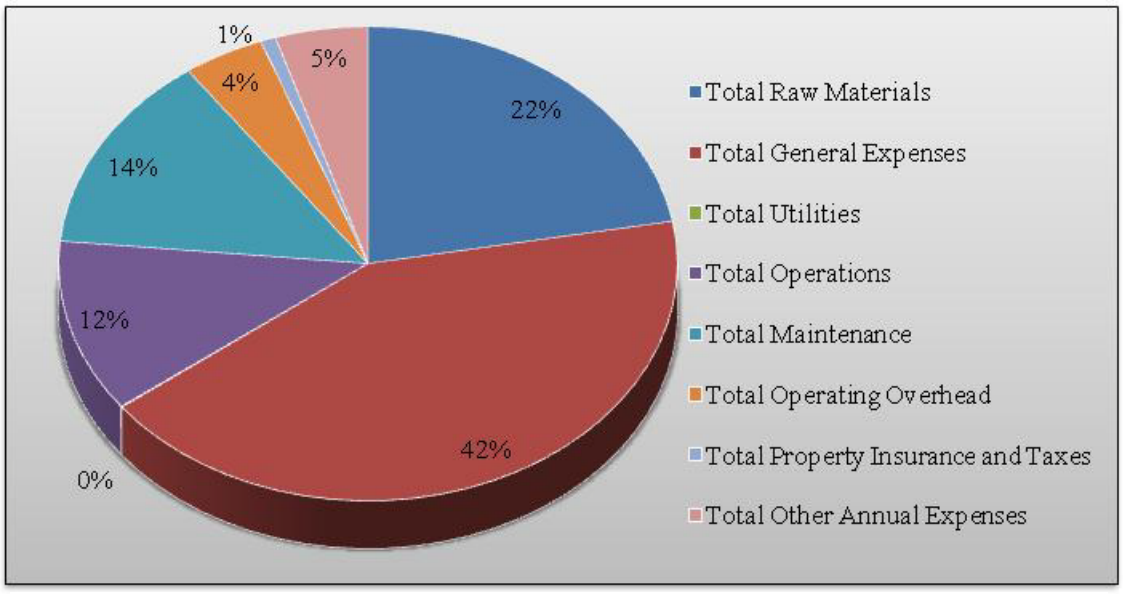

Figure 3-24 Chip Cost Composition

In addition, there are overhead expenses that are associated with the operation as well. This can be broken down to 1) general plant overhead, 2) mechanical department services, 3) employee relations department, and 4) business services. These expense items are rather self-explanatory or previously addressed, and 7.1%, 2.4%, 5.9%, and 7.4% of the sum of DWB and MWB, respectively.

Rental fee for factory and office space is computed based on cost per square feet and estimated size of our plant:

$$\boldsymbol{Annual\ Rental\ Fee} = \frac{\boldsymbol{Cost}}{\boldsymbol{ft^2}} \times \boldsymbol{office}\ \&\ factory\ space \quad \textbf{3-34)}$$

The fixed cost components are tabulated in Table 3-9.

Fixed Cost Summary

April, 2009

MCCRA

			TOTAL
Operations			
	Direct Wages and Benefits:	$1,326,000	
	Direct Salaries and Benefits:	$198,900	
	Operating Supplies and Services:	$79,560	
	Total Operations:	**$1,604,460**	**$1,604,460**
Maintenance			
	Wages and Benefits:	$803,710	
	Salaries and Benefits:	$200,927	
	Materials and Services:	$803,710	
	Maintenance Overhead:	$40,185	
	Total Maintenance:	**$1,848,532**	**$3,452,992**
Operating Overhead			
	General Plant Overhead:	$179,597	
	Mechanical Department Services:	$60,709	
	Employee Relations Department:	$149,243	
	Business Services:	$187,186	
	Total Operating Overhead:	**$576,735**	**$4,029,727**
Property Insurance and Taxes			
	Property Insurance and Taxes:	$107,161	
	Total Property Insurance and Taxes:	**$107,161**	**$4,136,888**
Other Annual Expenses			
	Rent:	$510,000	
	Annual Licensing Fees:	$100,000	
	Miscellaneous:	$50,000	
	Total Other Annual Expenses:	**$660,000**	**$4,796,888**
TOTAL			**$4,796,888**

Table 3-9: A summary of fixed cost

3.5.3 Valuation/Discounted Cash Flow

The fundamental principle of the time valuation of money tells us that a dollar today is not the same as a dollar a year from today, simply because if we invest a dollar today in the market, we will get back a dollar plus interest. Similarly, a dollar a year from today is worth actually less than a dollar today. It is clear that the money values at different times are somehow related. This seemingly intricate relationship is unexpectedly simple; all cash flows at different times can be jointly expressed, that is, in the form of the discount interest rate [52]:

$$\boldsymbol{NPV = C_0 + \frac{C_1}{(1+r)} + \frac{C_2}{(1+r)^2} + \cdots + \frac{C_t}{(1+r)^t}} \qquad \textbf{(3-35)}$$

where C subscript represents the cash flow at date 0 (or today), date 1, date 2 and so on. The net present value (NPV) is the sum of discounted cash flows; in another words, paying one all these cash amounts (C_0, C_1...C_t) at different times is equivalent to paying him/her a lumped sum of NPV today. Therefore, once we know the discount rate, we can express our project sales and costs in terms of money today.

Simple as it seems, estimation of the discount rate is one of the major challenges in financial analysis. Often, the discount rate is misinterpreted as simply the interest rate in the bank, while in reality it is the expected return on the asset. For this reason, we will expound on how we obtain the discount rate through the capital asset pricing model (CAPM):

$$\boldsymbol{\bar{R} = R_F + \beta \times (\bar{R}_M - R_F)} \qquad \textbf{(3-36)}$$

$\bar{R}$ is the expected return on our project (taken as the discount rate). R_F represents the risk free expected rate of return or the current return on 10-year treasury bills, which is 2.7%. [53] $\bar{R}_M$ is the expected return on the market and estimated to be 10.83% based on the Dow Jones

Industry Average from 1975 to 2006.[54] The difference between $\bar{R}_M$ and R_F, 8.13%, known as the market risk premium, is always positive because average return on the market is higher than riskless interest rates historically. β is a measure of risk or volatility on return associated with our project. The exact definition can be clarified by this equation:

$$\boldsymbol{\beta = \frac{Cov(R_i, R_M)}{\sigma^2(R_M)}} \quad \textbf{3-37)}$$

The numerator is the covariance between return on a similar project and average return on the market, while the denominator is the variance of the average return on the market. We selected few biotech startup companies from the market and take the average of their beta values to give a crude estimate of the beta value for our project.[55] Given all the required inputs into Table 3-11, the discount rate is then calculated to be about 15%. Now, we have all the information we need to construct the cash flow table; in summary, the required parameters are initial capital investment, variable cost/year, fixed cost/year and the discount rate. The net present value (NPV) and investor rate of return (IRR) are also included in Table 3-11.

Stock	Beta
Arrhythmia Research Technology, Inc.	1.22
American Shared Hospital Services	1.73
Advocat Inc.	1.33
Health Fitness Corporation	1.47
Synergetics USA Inc.	1.52
MTS Medication Technologies, Inc.	1.20
Dialysis Corporation of America	1.45
Bio-Imaging Technologies, Inc.	1.63
Average	**1.43**

Table 3-10: beta estimation from similar stocks

Cash Flow Summary

April, 2009

MCCRA

Year	Percentage of Design Capacity	Sales	Capital Costs	Working Capital	Variable Costs	Fixed Costs	Depreciation Allowance	Depletion Allowance	Taxable Income	Income Tax Costs	Net Earnings	Annual Cash Flow	Cumulative Net Present Value at 15.0%
2010	0.0%	Design	-$93,000									-$93,000	-$93,000
2011	0.0%	Construction	-$2,500,000									-$2,500,000	-$2,266,900
2012	0.0%	Construction	-$2,500,000	-$1,200,000								-$3,700,000	-$5,064,600
2013	20.0%	$10,000,000	-$273,500	-$2,400,000	-$1,758,400	-$4,796,900	$765,667	$0	$4,210,367	-$1,557,800	$2,652,567	-$786,600	-$5,581,800
2014	60.0%	$30,000,000	$0	-$2,400,000	-$5,275,300	-$4,796,900	$1,312,190	$0	$21,239,990	-$7,858,800	$13,381,190	$9,669,000	-$53,500
2015	100.0%	$50,000,000	$0		-$8,792,200	-$4,796,900	$937,126	$0	$37,348,026	-$13,818,800	$23,529,226	$22,592,100	$11,178,800
2016	100.0%	$50,000,000	$0		-$8,792,200	-$4,796,900	$669,222	$0	$37,080,122	-$13,719,600	$23,360,522	$22,691,300	$20,988,900
2017	100.0%	$50,000,000	$0		-$8,792,200	-$4,796,900	$478,475	$0	$36,889,375	-$13,649,100	$23,240,275	$22,761,800	$29,545,900
2018	100.0%	$50,000,000	$0		-$8,792,200	-$4,796,900	$477,939	$0	$36,888,839	-$13,648,900	$23,239,939	$22,762,000	$36,986,800
2019	100.0%	$50,000,000	$0	$6,000,000	-$8,792,200	-$4,796,900	$478,475	$0	$36,889,375	-$13,649,100	$23,240,275	$28,761,800	$45,162,700

Profitability Measures

April, 2009

MCCRA

The Investor's Rate of Return (IRR) for this Project is: **91.24%**

The Net Present Value (NPV) at 15% for this Project is: **$45,162,700**

ROI Analysis (Third Production Year)

Annual Sales:	$50,000,000
Annual Costs:	-$13,589,100
Depreciation:	-$428,600
Income Tax:	-$13,313,500
Net Earnings:	$23,097,400
Total Capital Investment:	$11,366,500
ROI:	**203.2%**

Table 3-11: Cash Flow and Profitability Summary

3.5.4 Sensitivity Analyses

Although a price estimate of the cost of the fabrication of each chip has been done to the best of our ability, price fluctuation in the future is not something that is readily accounted for. It is anticipated that some of the cost estimates based on the current state of the economy might not be consistent throughout the operating life of our plant.

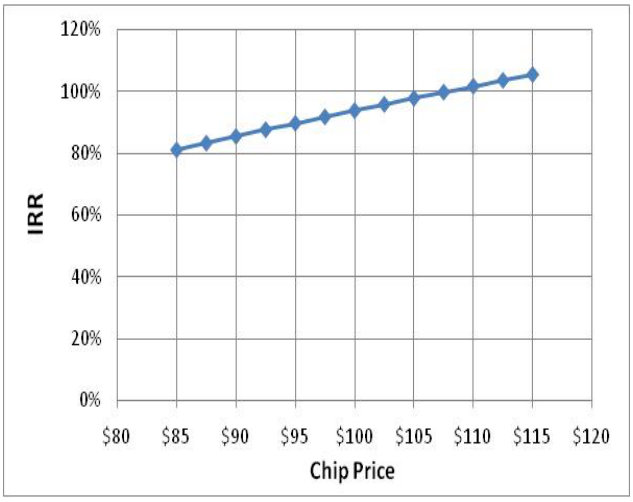

Figure 3-25: Sensitivity Analysis: Chip Price vs. IRR

For example, some of the raw material prices might change; the chip price might need to be adjusted to respond to our competitors; gas price might go up due to short supply, increasing transportation cost. Uncertainty clouds the future in every way, and it is generally common practice to observe how much each cost/revenue component causes the investor rate of return (IRR) to change. In another words, we want to see how "resistant" IRR is in response to "disturbances" in fixed cost, variable costs, product price and initial capital investment. Amongst all of them, variable cost and product price are most likely subject to change. Figure 3-25 shows the IRR at chip price ranging from $85 to $115. The steepness

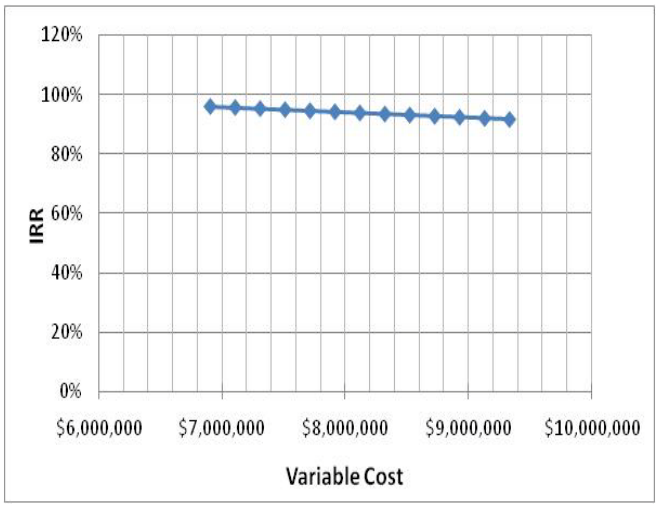

Figure 3-26 Sensitivity Analysis: Variable Cost vs. IRR.

of the slope gives us an idea how sensitive IRR is in response to potential changes; the closer to be horizontal implies the more stable IRR. Similarly, IRR versus variable cost is graphed in Figure 3-26; the slope interpretation still holds. IRR sensitivity analyses with changes in initial investment and fixed cost are not discussed here because it is anticipated that unexpected changes to these two parameters are unlikely. However, a full IRR sensitivity analysis for all 4 parameters is included in the appendix for completion.

3.5.5 Capital Structure

Once the capital budgeting is complete, a decision on how to finance the project needs to be made. There are generally two sources of financing: equity and debt. If a combination of the two is used in our project, dividends are paid to stock holders while interest is also paid out annually to bond holders. Because interest pay is tax deductable and dividend is not, there is an asymmetry in the tax code that enables us to increase the NPV of our company by borrowing money to finance our project. It should be noted that so far we assume 100% ownership of the project. However, there is also risk associated with debt, most notably the risk of bankruptcy. For these reasons, there must be an optimum equity to debt ratio that maximizes the value of our company. The follow equation helps explain this concept:

$$V_L = V_U + PVTS - PVCFD$$

where V_L is the value of the company with leverage; PVTS represents the present value of tax reduction due to debt financing; and PVCFD is the present value of cost of financial distress (CFD).

Numerical calculations will not be performed in regard to capital structure. The purpose of this section is to qualitatively describe the value increase potential of our project and the type of decisions we need to make beyond the preliminary design stage.

3.5.6 Conclusions

For 100% ownership of the project, the resulted net present value (NPV) of about $45 million looks promising. The investor's rate of return (IRR) of about 91% is extremely lucrative and seemingly unreasonable. However, it should be noted that the initial investment for this project is relatively small compared to the cash inflow from sales. For example, the total capital investment associated with the chip manufacture is about 11 million while the annual sales are $50 million. The IRR reflects the ratio of profit to the initial investment. Generally, IRR is not used as the only guideline to accept or reject mutually exclusive projects because it neglects the magnitude of the investment. On the other hand, a positive NPV suggests that the project not only satisfies the required return of 15% (r=15%) but also gives an idea of how much extra value the project adds in addition to the required return. Hence, we should accept this project with a positive NPV value of $45 million.

Although the NPV of the project indicates profitability in the long run, the initial capital investment and the FDA approval costs at the first 3 years represent a huge cash outflow at the beginning. Table 3-11 (Cash Flow Table) suggests the payback period is 6 years, after which the initial value put into the project is starts to be regained. In reality, a project with this type of cash flow structure represents a risky investment. Nonetheless, for a biomedical device development firm, this is rather typical and unavoidable.

Sensitivity analyses are carried out on IRR with two and single inputs variation from the expected value. The resulted IRR ranges from 75% to 110% (Appendix 7.7.4), which looks favorable because of its implication that our project IRR is not susceptible to deviation from expected sales or costs of operation.

The financial analysis in this report is performed by assuming 100% ownership. In reality, this might not be actually feasible. Presumably, the project is financed with equity and debt through venture capital. The capital structure with debt reduces our tax liability, thus increases the value of our company.

Limitations have certainly been encountered during calculations of the financials. For instance, the quotes obtained from raw material and equipment suppliers might not be accurate because the sales representatives might not be aware of the magnitude of our intended purchase, the discount rate estimated by the capital asset price model might represent an underestimation of risk involved with our project, the expected market share might not be as optimistic as 50%; or there might be some competitors that we are not aware of. If this project is carried out beyond the preliminary design stage, these are some areas that needed to be closely examined.

Part 4 **Development, Manufacturing, and Product-Introduction Stages**

4.1 FDA approval

Since the MCCRA System is considered to be a medical device, it is necessary to obtain FDA approval before distribution to hospitals can begin. One of the first steps is verify the class of our device. Since the system has multiple components and is responsible for giving an accurate reading that will in turn be translated into a medication dosing amount, it will not fall into the category of Class I devices. Class I encapsulates basic medical instruments, such as tongue depressors. The proper functioning of these devices can mostly be determined by basic inspection, and their failure is either relatively impossible or presents no potential harm to the patient. However, Class III devices are those which if they were to fail, could be harmful or even life-threatening, such as pacemakers. The MCCRA System seems to fall in the category of a Class II device. It has the potential for failure and its efficiency/safety must be demonstrated, but any malfunction of the device is not life-threatening to the patient. Determination of the class of the medical device is important in determining what paperwork needs to be filled out as well as what types of trials need to take place in order to gain FDA approval. Product design specifications, manufacturing protocols, and product prototypes must be submitted to the FDA in order for verification of the MCCRA System as a Class II device. Once this verification is obtained, the necessary paperwork can begin to be filled out and submitted and clinical trials can begin. [3]

4.1.1 Clinical Trials-[56]

In order to gain FDA approval, it will need to be proved that the MCCRA System is both safe and effective. One step is to try to prove Substantial Equivalence. This means

that it will need to be shown that the basic mechanics of operation are the same as a product that is already on the market, and therefore can already be assumed to have a similar level of safeness and effectiveness.

The safeness and effectiveness of the MCCRA System will also be tested through a series of clinical trials. In each Phase of the trials, every test subject will first be tested without any additional medication, to serve as a control for that individual. Then testing will be done with various amounts of clopidogrel dosing.

4.1.1.1 Phase I

In Phase I, approximately 75 test subjects will be used. Each subject will tested at two different times. The first test will be run without giving any amount of medication to the test subject. About a week later, the subject will be given a standard pre-angioplasty dose of clopidogrel (300 mg [57]) and his blood will be reanalyzed the next day. This data will be compiled and analyzed. The purpose of Phase I will primarily be to prove that the MCCRA System is in fact safe and does show a variance in detection data as a result of clopidogrel administration. It is estimated that this phase will take approximately 1 month to run and analyze.

4.1.1.2 Phase II

In Phase II, the number of test subjects will be increase four-fold to approximately 300. In this series of testing, each subject will be tested 3 times. The first test will be run without giving any amount of medication to the test subject. About one week later, the subject will be administered one half of a standard dose of clopidogrel (150 mg) and his blood will be tested the following day. Another two weeks later, the subject will be given a

full standard dose of clopidogrel (300 mg [57]), and his blood will be tested the next day. All of these results will be compiled and analyzed. The primary intention of Phase II will be to start proving the effectiveness of the device by demonstrating the sensitivity of the testing (i.e. the variance of results due to variance of dosing amount) as well as to further prove the safety of the device and procedure. It is estimated that this phase of trials and data analysis will take approximately 5 months to complete.

4.1.1.3 Phase III

Phase III the number of test subjects will increase ten-fold from Phase II, to approximately 3,000. In this phase, each subject will be tested four separate times. The subject will first be tested before the administering of any amount of medication. One week later, the subject will be administered one-quarter of the standard clopidogrel dose (75 mg) and will be tested the next day. This will be repeated two weeks later for a one-half standard dose (150 mg) and then two weeks after that for a full standard dose (300 mg). Once again the data will be compiled and analyzed. The purpose of Phase III is to prove that the device is ready for distribution to hospitals. The estimated duration of this phase is one year.

*4.1.1.4 Phase IV**

(N.B. Phase IV* is going to be denoted with an asterisk because although it is technically a continuation of the clinical research, this phase actually takes place after FDA approval has been obtained.) In Phase IV*, one MCCRA device and 25 disposable chips are distributed (at no cost) to each of 50 hospitals nation-wide (the hospitals that receive the free box and chips are the 50 hospitals with the highest number of angioplasties performed per year in the United States.) By targeting the largest hospitals, our company has a better

chance of capturing a large amount of the market. In addition, the hospitals that receive these boxes will do so under the obligation of providing our company with feedback. By analyzing the data of a bigger and broader range of patients, any possible variances that may only occur in a small portion of the population may be discovered. The purpose of Phase IV* is to investigate both this possibility, and also as a marketing tool to get the MCCRA System into hospitals.

4.1.2 Manufacturing

In order to perform the clinical trials, the manufacturing facility must be operating at partial capacity in order to produce the needed chips. In order to determine this capacity, the number of MCCRA chips that will be required are tabulated in Table 4-1.

	NUMBER OF SUBJECTS REQUIRED	CHIPS REQUIRED FOR TESTING	EXTRA CHIPS REQUIRED (25%)	TOTAL CHIPS REQUIRED	TIME FRAME
PHASE I	75	150	50	200	1 month
PHASE II	300	900	300	1,200	5 months
PHASE III	3,000	12,000	4,000	16,000	12 months
TOTALS	**3,380**	**13,050**	**4,350**	**17,400**	**18 months**

Table 4-1 Number of test subjects and chips required for each phase of the clinical trials, as well as the approximated duration of each testing phase. The chips required for testing are based on the number of chips that are to be used per test subject in each phase (Phase I = 2 chips per subject, Phase II = 3 chips per subject, Phase III = 4 chips per subject). The extra chips that are factored in are to account for Quality Assurance Testing (1 chip tested per 4 chips produced).

The total number of chips required (with Quality Assurance Testing factored in) is 17,400. The rate of assumed chip failure must also be factored into this total (i.e. assume that 1 chip fails due to defect for every 4 chips that are produced, or a 25% failure rate). (See Equation 4-1)

$$\frac{\mathbf{17{,}400}\ \boldsymbol{non\text{-}defect\ chips}}{\mathbf{75\%}\ \boldsymbol{of\ chip\ production}} = \frac{\mathbf{23{,}200}\ \boldsymbol{total\ chips}}{\mathbf{100\%}\ \boldsymbol{of\ chip\ production}} \qquad \textbf{(4-1)}$$

This means that at least 23,200 chips must be manufactured in order to accommodated those needed in the clinical trials. Assuming that these are all produced in the first year of manufacturing (so that production of chips does not hold up the execution of the trials), the operation of the facility must be functioning at 4.2% of full operating capacity (See Equation 4-2).

$$\frac{\mathbf{23{,}200}\ \boldsymbol{chips\ needed\ per\ year}}{\mathbf{550{,}000}\ \boldsymbol{chips\ made\ per\ year\ at\ full\ op}} = \mathbf{4.2\%}\ \boldsymbol{of\ full\ operation} \quad \textbf{(4-2)}$$

For ease of calculation, this is approximated at 5% of the manufacturing facility's full operating capacity (or about 200 chips produced per day). In the next year, during which the FDA approval process will still be underway, the facility will continue to operate at 5% of full capacity. This will build up an inventory of MCCRA chips that will be stored and used for distribution in the following year, enabling the shipment of chips to the 50 hospitals that will receive free equipment right after FDA approval has been obtained, rather than needing to delay distribution until fabrication of the chips is completed.

4.2 Recommendations for Future Studies

By utilizing the microfluidics technology, we allow ourselves to study one of the most promising and intense areas of research. As new applications of this concept is discovered, constant adaptation of our device is essential to keep pace with our competitors.

One of the advantages of our device is its expandability to other areas of applications by simple modifications. For example, MRS 2395 could simply be replaced with its aspirin counterpart (another anti-coagulant), so patients' resistance to aspirin can also be measured. Of course, mixing behavior of aspirin is to be characterized and the dissolution of it should be studied; the box is being re-customized.

Clopidogrel's usage has a potential to grow as its benefits are becoming known to the rest of the world. Marketed in 110 countries, clopidogrel sales is currently ranked 2nd worldwide and exhibiting a growth rate of 20%, fueling for demand for clopidogrel resistance assay soon enough. Although the US is our major target market at this stage, we should tailor a financial strategy to capture our share of the international market in a timely fashion.

In this design report, the box manufacture is out sourced to an electronic supplier, who makes a 30% profit by the synergy from sales of our chips. Rather than relying on an out source supplier, we can set up a box manufacturing division in our own site, which would facilitate the constant research and development aided improvements on our technology.

Part 5 **Acknowledgements**

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Part 7 **Appendix**

7.1 MSDS Reports

7.1.1 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 develo ps, 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; UnspecifiedFish: 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 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# 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.

7.1.2 MRS 2395

SIGMA-ALDRICH

Material Safety Data Sheet

Version 3.0
Revision Date 12/25/2008
Print Date 04/08/2009

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	MRS 2395
Product Number	:	M5942
Brand	:	Sigma
Company	:	Sigma-Aldrich 3050 Spruce Street SAINT LOUIS MO 63103 USA
Telephone	:	+1 800-325-5832
Fax	:	+1 800-325-5052
Emergency Phone #	:	(314) 776-6555

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms	:	2,2-Dimethyl-propionic acid 3-(2-chloro-6-methylaminopurin-9-yl)-2-(2,2-dimethyl-propionyloxymethyl)-propyl ester
Formula	:	$C_{20}H_{30}ClN_5O_4$
Molecular Weight	:	439.94 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
MRS 2395			
491611-55-3	-	-	-

3. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards
No known OSHA hazards

HMIS Classification
Health Hazard: 0
Flammability: 0
Physical hazards: 0

NFPA Rating
Health Hazard: 0
Fire: 0
Reactivity Hazard: 0

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Ingestion	May be harmful if swallowed.

4. FIRST AID MEASURES

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

5. FIRE-FIGHTING MEASURES

Flammable properties

Flash point	no data available
Ignition temperature	no data available

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Avoid dust formation.

Environmental precautions
Do not let product enter drains.

Methods for cleaning up
Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Handling
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Storage
Keep container tightly closed in a dry and well-ventilated place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
For prolonged or repeated contact use protective gloves.

Eye protection
Safety glasses

Hygiene measures
General industrial hygiene practice.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid

Safety data

pH	no data available
Melting point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Storage stability
Stable under recommended storage conditions.

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas

11. TOXICOLOGICAL INFORMATION

Acute toxicity

no data available

Irritation and corrosion

no data available

Sensitisation

no data available

Chronic exposure

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Signs and Symptoms of Exposure

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be harmful if swallowed.

12. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)

no data available

Ecotoxicity effects

no data available

Further information on ecology

no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

OSHA Hazards
No known OSHA hazards

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
MRS 2395	491611-55-3

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
No SARA Hazards

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
MRS 2395	491611-55-3	

New Jersey Right To Know Components

	CAS-No.	Revision Date
MRS 2395	491611-55-3	

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth, or any other reproductive defects.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

7.1.3 Collagen

Health	2
Fire	1
Reactivity	0
Personal Protection	E

Material Safety Data Sheet
Collagen MSDS

Section 1: Chemical Product and Company Identification

Product Name: Collagen

Catalog Codes: SLC2137

CAS#: 9007-34-5

RTECS: Not available.

TSCA: TSCA 8(b) inventory: Collagen

CI#: Not available.

Synonym: Collagen (Insoluble)

Chemical Name: Collagen

Chemical Formula: Not available.

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call:
1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Collagen	9007-34-5	100

Toxicological Data on Ingredients: Collagen LD50: Not available. LC50: Not available.

Section 3: Hazards Identification

Potential Acute Health Effects: Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation.

Potential Chronic Health Effects:
Slightly hazardous in case of skin contact (sensitizer).
CARCINOGENIC EFFECTS: Not available.
MUTAGENIC EFFECTS: Not available.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Skin Contact:
In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Serious Inhalation: Not available.

Ingestion:
Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: May be combustible at high temperature.

Auto-Ignition Temperature: Not available.

Flash Points: Not available.

Flammable Limits: Not available.

Products of Combustion: Not available.

Fire Hazards in Presence of Various Substances:
Slightly flammable to flammable in presence of heat.
Non-flammable in presence of shocks.

Explosion Hazards in Presence of Various Substances:
Slightly explosive in presence of open flames and sparks.
Non-explosive in presence of shocks.

Fire Fighting Media and Instructions:
SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

Special Remarks on Fire Hazards: As with most organic solids, fire is possible at elevated temperatures

Special Remarks on Explosion Hazards:
Fine dust dispersed in air in sufficient concentrations, and in the presences of an ignition source is a potential dust explosion hazard.

Section 6: Accidental Release Measures

Small Spill:
Use appropriate tools to put the spilled solid in a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.

Large Spill:
Use a shovel to put the material into a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.

Section 7: Handling and Storage

Precautions:
Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If you feel unwell, seek medical attention and show the label when possible. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents.

Storage:
Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 8°C (46.4°F). Refrigerate

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

Personal Protection:
Splash goggles. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits: Not available.

Section 9: Physical and Chemical Properties

Physical state and appearance: Solid.

Odor: Not available.

Taste: Not available.

Molecular Weight: Not available.

Color: Not available.

pH (1% soln/water): Not applicable.

Boiling Point: Not available.

Melting Point: Not available.

Critical Temperature: Not available.

Specific Gravity: Not available.

Vapor Pressure: Not applicable.

Vapor Density: Not available.

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: Not available.

Ionicity (in Water): Not available.

Dispersion Properties: Not available.

Solubility:
Insoluble in cold water, hot water.
Solubility in 0.5 M Acetic Acid: 1 mg/ml

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Excess heat, dust generation, incompatible materials

Incompatibility with various substances: Reactive with oxidizing agents.

Corrosivity: Not available.

Special Remarks on Reactivity: Not available.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Inhalation. Ingestion.

Toxicity to Animals:
LD50: Not available.
LC50: Not available.

Chronic Effects on Humans: Not available.

Other Toxic Effects on Humans: Hazardous in case of skin contact (irritant), of ingestion, of inhalation.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans: Not available.

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects:
Skin: May cause skin irritation with severe redness and moderate raising of skin.
Eyes: May cause eye irritation.
Inhalation: May cause respiratory tract irritation.
Ingestion: May cause gastrointestinal tract irritation with nausea, and vomiting. Other symptoms may include headache, dizziness, and tiredness and unconciousness
The toxicological properties of this substance have not been fully investigated.
Chronic Potential Health Effects:

Skin: May cause allergic skin reaction

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: Not available.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: Not a DOT controlled material (United States).

Identification: Not applicable.

Special Provisions for Transport: Not applicable.

Section 15: Other Regulatory Information

Federal and State Regulations: TSCA 8(b) inventory: Collagen

Other Regulations: EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada): Not controlled under WHMIS (Canada).

DSCL (EEC):
R36/38- Irritating to eyes and skin.
S2- Keep out of the reach of children.
S46- If swallowed, seek medical advice immediately and show this container or label.

HMIS (U.S.A.):

Health Hazard: 2

Fire Hazard: 1

Reactivity: 0

Personal Protection: E

National Fire Protection Association (U.S.A.):

Health: 1

Flammability: 1

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves.
Lab coat.
Dust respirator. Be sure to use an approved/certified respirator or equivalent.
Splash goggles.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

Created: 10/09/2005 04:58 PM

Last Updated: 11/06/2008 12:00 PM

7.1.4 ADP

ScienceLab.com
Chemicals & Laboratory Equipment

Health	1
Fire	1
Reactivity	0
Personal Protection	E

Material Safety Data Sheet
Adenosine-5'-diphosphate disodium salt MSDS

Section 1: Chemical Product and Company Identification

Product Name: Adenosine-5'-diphosphate disodium salt

Catalog Codes: SLA3181

CAS#: 16178-48-6

RTECS: AU7467000

TSCA: TSCA 8(b) inventory: Adenosine-5'-diphosphate disodium salt

CI#: Not available.

Synonym:

Chemical Name: Not available.

Chemical Formula: C10H13N5O10P2Na2.2H2O

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call:
1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Adenosine-5'-diphosphate disodium salt	16178-48-6	100

Toxicological Data on Ingredients: Not applicable.

Section 3: Hazards Identification

Potential Acute Health Effects:
Hazardous in case of ingestion. Slightly hazardous in case of skin contact (irritant), of eye contact (irritant), of inhalation.

Potential Chronic Health Effects:
Hazardous in case of ingestion.
Slightly hazardous in case of skin contact (irritant), of eye contact (irritant), of inhalation.
CARCINOGENIC EFFECTS: Not available.
MUTAGENIC EFFECTS: Not available.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if irritation occurs.

Skin Contact: Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops.

Serious Skin Contact: Not available.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Serious Inhalation: Not available.

Ingestion:
Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: May be combustible at high temperature.

Auto-Ignition Temperature: Not available.

Flash Points: Not available.

Flammable Limits: Not available.

Products of Combustion: These products are carbon oxides (CO, CO2), nitrogen oxides (NO, NO2...), phosphates. Some metallic oxides.

Fire Hazards in Presence of Various Substances: Not available.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available.
Risks of explosion of the product in presence of static discharge: Not available.

Fire Fighting Media and Instructions:
SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

Special Remarks on Fire Hazards: Not available.

Special Remarks on Explosion Hazards: Not available.

Section 6: Accidental Release Measures

Small Spill:
Use appropriate tools to put the spilled solid in a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.

Large Spill:
Use a shovel to put the material into a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.

Section 7: Handling and Storage

Precautions:
Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust.

Storage: Keep container tightly closed. Keep container in a cool, well-ventilated area.

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

Personal Protection: Safety glasses. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits: Not available.

Section 9: Physical and Chemical Properties

Physical state and appearance: Solid.

Odor: Not available.

Taste: Not available.

Molecular Weight: 507.2 g/mole

Color: Not available.

pH (1% soln/water): Not available.

Boiling Point: Not available.

Melting Point: Decomposes.

Critical Temperature: Not available.

Specific Gravity: Not available.

Vapor Pressure: Not applicable.

Vapor Density: Not available.

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: Not available.

Ionicity (in Water): Not available.

Dispersion Properties: Not available.

Solubility: Not available.

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Not available.

Incompatibility with various substances: Not available.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity: Not available.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Ingestion.

Toxicity to Animals:
LD50: Not available.
LC50: Not available.

Chronic Effects on Humans: Not available.

Other Toxic Effects on Humans:
Hazardous in case of ingestion.
Slightly hazardous in case of skin contact (irritant), of inhalation.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans: Not available.

Special Remarks on other Toxic Effects on Humans: Not available.

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The products of degradation are more toxic.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:

Section 14: Transport Information

DOT Classification: Not a DOT controlled material (United States).

Identification: Not applicable.

Special Provisions for Transport: Not applicable.

Section 15: Other Regulatory Information

Federal and State Regulations: TSCA 8(b) inventory: Adenosine-5'-diphosphate disodium salt

Other Regulations: Not available.

Other Classifications:

WHMIS (Canada): Not controlled under WHMIS (Canada).

DSCL (EEC):
This product is not classified according to the EU regulations.

HMIS (U.S.A.):

Health Hazard: 1

Fire Hazard: 1

Reactivity: 0

Personal Protection: E

National Fire Protection Association (U.S.A.):

Health: 1

Flammability: 1

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves.
Lab coat.
Dust respirator. Be sure to use an approved/certified respirator or equivalent.
Safety glasses.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

Created: 10/09/2005 03:37 PM

Last Updated: 11/06/2008 12:00 PM

7.1.5 Isopropanol

Material Safety Data Sheet
Isopropyl alcohol MSDS

Section 1: Chemical Product and Company Identification

Product Name: Isopropyl alcohol

Catalog Codes: SLI1153, SLI1579, SLI1906, SLI1246, SLI1432

CAS#: 67-63-0

RTECS: NT8050000

TSCA: TSCA 8(b) inventory: Isopropyl alcohol

CI#: Not available.

Synonym: 2-Propanol

Chemical Name: isopropanol

Chemical Formula: C3-H8-O

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call: 1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Isopropyl alcohol	67-63-0	100

Toxicological Data on Ingredients: Isopropyl alcohol: ORAL (LD50): Acute: 5045 mg/kg [Rat]. 3600 mg/kg [Mouse]. 6410 mg/kg [Rabbit]. DERMAL (LD50): Acute: 12800 mg/kg [Rabbit].

Section 3: Hazards Identification

Potential Acute Health Effects:
Hazardous in case of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (irritant, sensitizer, permeator).

Potential Chronic Health Effects:
Slightly hazardous in case of skin contact (sensitizer).
CARCINOGENIC EFFECTS: A4 (Not classifiable for human or animal.) by ACGIH, 3 (Not classifiable for human.) by IARC.
MUTAGENIC EFFECTS: Not available.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Classified Reproductive system/toxin/female, Development toxin [POSSIBLE].
The substance may be toxic to kidneys, liver, skin, central nervous system (CNS).
Repeated or prolonged exposure to the substance can produce target organs damage.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention.

Skin Contact:
Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops. Cold water may be used.

Serious Skin Contact: Not available.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

Serious Inhalation:
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

Ingestion:
Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: Flammable.

Auto-Ignition Temperature: 399°C (750.2°F)

Flash Points: CLOSED CUP: 11.667°C (53°F) - 12.778 deg. C (55 deg. F) (TAG)

Flammable Limits: LOWER: 2% UPPER: 12.7%

Products of Combustion: These products are carbon oxides (CO, CO2).

Fire Hazards in Presence of Various Substances:
Highly flammable in presence of open flames and sparks, of heat.
Flammable in presence of oxidizing materials.
Non-flammable in presence of shocks.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available.
Explosive in presence of open flames and sparks, of heat.

Fire Fighting Media and Instructions:
Flammable liquid, soluble or dispersed in water.
SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use alcohol foam, water spray or fog.

Special Remarks on Fire Hazards:
Vapor may travel considerable distance to source of ignition and flash back. CAUTION: MAY BURN WITH NEAR INVISIBLE FLAME.
Hydrogen peroxide sharply reduces the autoignition temperature of Isopropyl alcohol.
After a delay, Isopropyl alcohol ignites on contact with dioxgenyl tetrafluorborate, chromium trioxide, and potassium tert-butoxide. When heated to decomposition it emits acrid smoke and fumes.

Special Remarks on Explosion Hazards:
Secondary alcohols are readily autooxidized in contact with oxygen or air, forming ketones and hydrogen peroxide.
It can become potentially explosive.
It reacts with oxygen to form dangerously unstable peroxides which can concentrate and explode during distillation or evaporation. The presence of 2-butanone increases the reaction rate for peroxide formation.
Explosive in the form of vapor when exposed to heat or flame. May form explosive mixtures with air.
Isopropyl alcohol + phosgene forms isopropyl chloroformate and hydrogen chloride.
In the presence of iron salts, thermal decompositon can occur, whicn in some cases can become explosive.
A homogeneous mixture of concentrated peroxides + isopropyl alcohol are capable of detonation by shock or heat.
Barium perchlorate + isopropyl alcohol gives the highly explosive alkyl perchlorates.
It forms explosive mixtures with trinitormethane and hydrogen peroxide.
It produces a violent explosive reaction when heated with aluminum isopropoxide + crotonaldehyde.
Mixtures of isopropyl alcohol + nitroform are explosive.

Section 6: Accidental Release Measures

Small Spill:
Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

Large Spill:
Flammable liquid.
Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not touch spilled material. Prevent entry into sewers, basements or confined areas; dike if needed. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Section 7: Handling and Storage

Precautions:
Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Avoid contact with eyes. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Keep away from incompatibles such as oxidizing agents, acids.

Storage:
Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

Personal Protection:
Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits:
TWA: 983 STEL: 1230 (mg/m3) [Australia]
TWA: 200 STEL: 400 (ppm) from ACGIH (TLV) [United States] [1999]

TWA: 980 STEL: 1225 (mg/m3) from NIOSH
TWA: 400 STEL: 500 (ppm) from NIOSH
TWA: 400 STEL: 500 (ppm) [United Kingdom (UK)]
TWA: 999 STEL: 1259 (mg/m3) [United Kingdom (UK)]
TWA: 400 STEL: 500 (ppm) from OSHA (PEL) [United States]
TWA: 980 STEL: 1225 (mg/m3) from OSHA (PEL) [United States]Consult local authorities for acceptable exposure limits.

Section 9: Physical and Chemical Properties

Physical state and appearance: Liquid.

Odor:
Pleasant. Odor resembling that of a mixture of
ethanol and acetone.

Taste: Bitter. (Slight.)

Molecular Weight: 60.1 g/mole

Color: Colorless.

pH (1% soln/water): Not available.

Boiling Point: 82.5°C (180.5°F)

Melting Point: -88.5°C (-127.3°F)

Critical Temperature: 235°C (455°F)

Specific Gravity: 0.78505 (Water = 1)

Vapor Pressure: 4.4 kPa (@ 20°C)

Vapor Density: 2.07 (Air = 1)

Volatility: Not available.

Odor Threshold:
22 ppm (Sittig, 1991)
700 ppm for unadapted panelists (Verschuren, 1983).

Water/Oil Dist. Coeff.: The product is equally soluble in oil and water; log(oil/water) = 0.1

Ionicity (in Water): Not available.

Dispersion Properties: See solubility in water, methanol, diethyl ether, n-octanol, acetone.

Solubility:
Easily soluble in cold water, hot water, methanol, diethyl ether, n-octanol, acetone.
Insoluble in salt solution.
Soluble in benzene.
Miscible with most organic solvents including alcohol, ethyl alcohol, chloroform.

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Heat, Ignition sources, incompatible materials

Incompatibility with various substances: Reactive with oxidizing agents, acids, alkalis.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity:
Reacts violently with hydrogen + palladium combination, nitroform, oleum, COCl2, aluminum triisopropoxide, oxidants
Incompatible with acetaldehyde, chlorine, ethylene oxide, isocyanates, acids, alkaline earth, alkali metals, caustics, amines, crotonaldehyde, phosgene, ammonia.
Isopropyl alcohol reacts with metallic aluminum at high temperatures.
Isopropyl alcohol attacks some plastics, rubber, and coatings.
Vigorous reaction with sodium dichromate + sulfuric acid.

Special Remarks on Corrosivity: May attack some forms of plastic, rubber and coating

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Absorbed through skin. Dermal contact. Eye contact. Inhalation.

Toxicity to Animals:
WARNING: THE LC50 VALUES HEREUNDER ARE ESTIMATED ON THE BASIS OF A 4-HOUR EXPOSURE.
Acute oral toxicity (LD50): 3600 mg/kg [Mouse].
Acute dermal toxicity (LD50): 12800 mg/kg [Rabbit].
Acute toxicity of the vapor (LC50): 16000 8 hours [Rat].

Chronic Effects on Humans:
CARCINOGENIC EFFECTS: A4 (Not classifiable for human or animal.) by ACGIH, 3 (Not classifiable for human.) by IARC.
DEVELOPMENTAL TOXICITY: Classified Reproductive system/toxin/female, Development toxin [POSSIBLE].
May cause damage to the following organs: kidneys, liver, skin, central nervous system (CNS).

Other Toxic Effects on Humans:
Hazardous in case of ingestion, of inhalation.
Slightly hazardous in case of skin contact (irritant, sensitizer, permeator).

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:
May cause adverse reproductive/teratogenic effects (fertility, fetoxicity, developmental abnormalities(developmental toxin)) based on animal studies.
Detected in maternal milk in human.

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects:
Skin: May cause mild skin irritation, and sensitization.
Eyes: Can cause eye irritation.
Inhalation: Breathing in small amounts of this material during normal handling is not likely to cause harmful effects. However, breathing large amounts may be harmful and may affect the respiratory system and mucous membranes (irritation), behavior and brain (Central nervous system depression - headache, dizziness, drowsiness, stupor, incoordination, unconciousness, coma and possible death), peripheral nerve and senstation, blood, urinary system, and liver.
Ingestion: Swallowing small amouts during normal handling is not likely to cause harmful effects. Swallowing large amounts may be harmful. Swallowing large amounts may cause gastrointestinal tract irritation with nausea, vomiting and diarrhea, abdominal pain. It also may affect the urinary system, cardiovascular system, sense organs, behavior or central nervous system (somnolence, generally depressed activity, irritability, headache, dizziness, drowsiness), liver, and respiratory system (breathing difficulty).
Chronic Potential Health Effects:

May cause defatting of the skin and dermatitis and allergic reaction.
May cause adverse reproductive effects based on animal data (studies).

Section 12: Ecological Information

Ecotoxicity: Ecotoxicity in water (LC50): 100000 mg/l 96 hours [Fathead Minnow]. 64000 mg/l 96 hours [Fathead Minnow].

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The product itself and its products of degradation are not toxic.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: CLASS 3: Flammable liquid.

Identification: : Isopropyl Alcohol UNNA: 1219 PG: II

Special Provisions for Transport: Not available.

Section 15: Other Regulatory Information

Federal and State Regulations:
Connecticut hazardous material survey.: Isopropyl alcohol
Illinois toxic substances disclosure to employee act: Isopropyl alcohol
Rhode Island RTK hazardous substances: Isopropyl alcohol
Pennsylvania RTK: Isopropyl alcohol
Florida: Isopropyl alcohol
Minnesota: Isopropyl alcohol
Massachusetts RTK: Isopropyl alcohol
New Jersey: Isopropyl alcohol
New Jersey spill list: Isopropyl alcohol
Director's list of Hazardous Substances: Isopropyl alcohol
Tennesee: Isopropyl alcohol
TSCA 8(b) inventory: Isopropyl alcohol
TSCA 4(a) final testing order: Isopropyl alcohol
TSCA 8(a) IUR: Isopropyl alcohol
TSCA 8(d) H and S data reporting: Isopropyl alcohol: Effective date: 12/15/86 Sunset Date: 12/15/96
TSCA 12(b) one time export: Isopropyl alcohol
SARA 313 toxic chemical notification and release reporting: Isopropyl alcohol

Other Regulations:
OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).
EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada):
CLASS B-2: Flammable liquid with a flash point lower than 37.8°C (100°F).
CLASS D-2B: Material causing other toxic effects (TOXIC).

DSCL (EEC):
R11- Highly flammable.
R36- Irritating to eyes.
S7- Keep container tightly closed.
S16- Keep away from sources of ignition - No smoking.
S24/25- Avoid contact with skin and eyes.
S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

HMIS (U.S.A.):

Health Hazard: 2

Fire Hazard: 3

Reactivity: 0

Personal Protection: h

National Fire Protection Association (U.S.A.):

Health: 1

Flammability: 3

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves.
Lab coat.
Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.
Splash goggles.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

Created: 10/09/2005 05:53 PM

Last Updated: 10/09/2005 05:53 PM

7.1.6 Acetone

Health	2
Fire	3
Reactivity	0
Personal Protection	H

Material Safety Data Sheet
Acetone MSDS

Section 1: Chemical Product and Company Identification

Product Name: Acetone

Catalog Codes: SLA3502, SLA1645, SLA3151, SLA3808

CAS#: 67-64-1

RTECS: AL3150000

TSCA: TSCA 8(b) inventory: Acetone

CI#: Not applicable.

Synonym: 2-propanone; Dimethyl Ketone; Dimethylformaldehyde; Pyroacetic Acid

Chemical Name: Acetone

Chemical Formula: C3-H6-O

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call: 1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Acetone	67-64-1	100

Toxicological Data on Ingredients: Acetone: ORAL (LD50): Acute: 5800 mg/kg [Rat]. 3000 mg/kg [Mouse]. 5340 mg/kg [Rabbit]. VAPOR (LC50): Acute: 50100 mg/m 8 hours [Rat]. 44000 mg/m 4 hours [Mouse].

Section 3: Hazards Identification

Potential Acute Health Effects:
Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator).

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: A4 (Not classifiable for human or animal.) by ACGIH.
MUTAGENIC EFFECTS: Not available.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Classified Reproductive system/toxin/female, Reproductive system/toxin/male [SUSPECTED].
The substance is toxic to central nervous system (CNS).
The substance may be toxic to kidneys, the reproductive system, liver, skin.
Repeated or prolonged exposure to the substance can produce target organs damage.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. Immediately flush eyes with running water for at least 15 minutes, keeping eyelids open. Cold water may be used. Get medical attention.

Skin Contact:
In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Cold water may be used.Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

Serious Inhalation:
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

Ingestion:
Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: Flammable.

Auto-Ignition Temperature: 465°C (869°F)

Flash Points: CLOSED CUP: -20°C (-4°F). OPEN CUP: -9°C (15.8°F) (Cleveland).

Flammable Limits: LOWER: 2.6% UPPER: 12.8%

Products of Combustion: These products are carbon oxides (CO, CO2).

Fire Hazards in Presence of Various Substances: Highly flammable in presence of open flames and sparks, of heat.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available.
Slightly explosive in presence of open flames and sparks, of oxidizing materials, of acids.

Fire Fighting Media and Instructions:
Flammable liquid, soluble or dispersed in water.
SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use alcohol foam, water spray or fog.

Special Remarks on Fire Hazards: Vapor may travel considerable distance to source of ignition and flash back.

Special Remarks on Explosion Hazards:
Forms explosive mixtures with hydrogen peroxide, acetic acid, nitric acid, nitric acid + sulfuric acid, chromic anydride, chromyl chloride, nitrosyl chloride, hexachloromelamine, nitrosyl perchlorate, nitryl perchlorate, permonosulfuric acid, thiodiglycol + hydrogen peroxide, potassium ter-butoxide, sulfur dichloride, 1-methyl-1,3-butadiene, bromoform, carbon, air, chloroform, thitriazylperchlorate.

Section 6: Accidental Release Measures

Small Spill:
Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

Large Spill:
Flammable liquid.
Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not touch spilled material. Prevent entry into sewers, basements or confined areas; dike if needed. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Section 7: Handling and Storage

Precautions:
Keep locked up.. Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, acids, alkalis.

Storage:
Store in a segregated and approved area (flammables area) . Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Keep away from direct sunlight and heat and avoid all possible sources of ignition (spark or flame).

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

Personal Protection:
Splash goggles. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits:
TWA: 500 STEL: 750 (ppm) from ACGIH (TLV) [United States]
TWA: 750 STEL: 1000 (ppm) from OSHA (PEL) [United States]
TWA: 500 STEL: 1000 [Austalia]
TWA: 1185 STEL: 2375 (mg/m3) [Australia]
TWA: 750 STEL: 1500 (ppm) [United Kingdom (UK)]
TWA: 1810 STEL: 3620 (mg/m3) [United Kingdom (UK)]
TWA: 1800 STEL: 2400 from OSHA (PEL) [United States]Consult local authorities for acceptable exposure limits.

Section 9: Physical and Chemical Properties

Physical state and appearance: Liquid.

Odor: Fruity. Mint-like. Fragrant. Ethereal

Taste: Pungent, Sweetish

Molecular Weight: 58.08 g/mole

Color: Colorless. Clear

pH (1% soln/water): Not available.

Boiling Point: 56.2°C (133.2°F)

Melting Point: -95.35 (-139.6°F)

Critical Temperature: 235°C (455°F)

Specific Gravity: 0.79 (Water = 1)

Vapor Pressure: 24 kPa (@ 20°C)

Vapor Density: 2 (Air = 1)

Volatility: Not available.

Odor Threshold: 62 ppm

Water/Oil Dist. Coeff.: The product is more soluble in water; log(oil/water) = -0.2

Ionicity (in Water): Not available.

Dispersion Properties: See solubility in water.

Solubility: Easily soluble in cold water, hot water.

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Excess heat, ignition sources, exposure to moisture, air, or water, incompatible materials.

Incompatibility with various substances: Reactive with oxidizing agents, reducing agents, acids, alkalis.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity: Not available.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Absorbed through skin. Dermal contact. Eye contact. Inhalation.

Toxicity to Animals:
WARNING: THE LC50 VALUES HEREUNDER ARE ESTIMATED ON THE BASIS OF A 4-HOUR EXPOSURE.
Acute oral toxicity (LD50): 3000 mg/kg [Mouse].
Acute toxicity of the vapor (LC50): 44000 mg/m3 4 hours [Mouse].

Chronic Effects on Humans:
CARCINOGENIC EFFECTS: A4 (Not classifiable for human or animal.) by ACGIH.

DEVELOPMENTAL TOXICITY: Classified Reproductive system/toxin/female, Reproductive system/toxin/male [SUSPECTED].
Causes damage to the following organs: central nervous system (CNS).
May cause damage to the following organs: kidneys, the reproductive system, liver, skin.

Other Toxic Effects on Humans:
Hazardous in case of skin contact (irritant), of ingestion, of inhalation.
Slightly hazardous in case of skin contact (permeator).

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:
May affect genetic material (mutagenicity) based on studies with yeast (S. cerevisiae), bacteria, and hamster fibroblast cells. May cause reproductive effects (fertility) based upon animal studies.
May contain trace amounts of benzene and formaldehyde which may cancer and birth defects. Human: passes the placental barrier.

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects:
Skin: May cause skin irritation. May be harmful if absorbed through the skin.
Eyes: Causes eye irritation, characterized by a burning sensation, redness, tearing, inflammation, and possible corneal injury.
Inhalation: Inhalation at high concentrations affects the sense organs, brain and causes respiratory tract irritation. It also may affect the Central Nervous System (behavior) characterized by dizzness, drowsiness, confusion, headache, muscle weakeness, and possibly motor incoordination, speech abnormalities, narcotic effects and coma. Inhalation may also affect the gastrointestinal tract (nausea, vomiting).
Ingestion: May cause irritation of the digestive (gastrointestinal) tract (nausea, vomiting). It may also affect the Central Nevous System (behavior), characterized by depression, fatigue, excitement, stupor, coma, headache, altered sleep time, ataxia, tremors as well at the blood, liver, and urinary system (kidney, bladder, ureter) and endocrine system. May also have musculoskeletal effects.
Chronic Potential Health Effects:
Skin: May cause dermatitis.
Eyes: Eye irritation.

Section 12: Ecological Information

Ecotoxicity:
Ecotoxicity in water (LC50): 5540 mg/l 96 hours [Trout]. 8300 mg/l 96 hours [Bluegill]. 7500 mg/l 96 hours [Fatthead Minnow]. 0.1 ppm any hours [Water flea].

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The product itself and its products of degradation are not toxic.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: CLASS 3: Flammable liquid.

Identification: : Acetone UNNA: 1090 PG: II

Special Provisions for Transport: Not available.

Section 15: Other Regulatory Information

Federal and State Regulations:
California prop. 65: This product contains the following ingredients for which the State of California has found to cause reproductive harm (male) which would require a warning under the statute: Benzene
California prop. 65: This product contains the following ingredients for which the State of California has found to cause birth defects which would require a warning under the statute: Benzene
California prop. 65: This product contains the following ingredients for which the State of California has found to cause cancer which would require a warning under the statute: Benzene, Formaldehyde
Connecticut hazardous material survey.: Acetone
Illinois toxic substances disclosure to employee act: Acetone
Illinois chemical safety act: Acetone
New York release reporting list: Acetone
Rhode Island RTK hazardous substances: Acetone
Pennsylvania RTK: Acetone
Florida: Acetone
Minnesota: Acetone
Massachusetts RTK: Acetone
Massachusetts spill list: Acetone
New Jersey: Acetone
New Jersey spill list: Acetone
Louisiana spill reporting: Acetone
California List of Hazardous Substances (8 CCR 339): Acetone
TSCA 8(b) inventory: Acetone
TSCA 4(a) final test rules: Acetone
TSCA 8(a) IUR: Acetone

Other Regulations:
OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).
EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada):
CLASS B-2: Flammable liquid with a flash point lower than 37.8°C (100°F).
CLASS D-2B: Material causing other toxic effects (TOXIC).

DSCL (EEC):
R11- Highly flammable.
R36- Irritating to eyes.
S9- Keep container in a well-ventilated place.
S16- Keep away from sources of ignition - No smoking.
S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

HMIS (U.S.A.):

Health Hazard: 2

Fire Hazard: 3

Reactivity: 0

Personal Protection: h

National Fire Protection Association (U.S.A.):

Health: 1

Flammability: 3

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves.
Lab coat.
Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.
Splash goggles.

Section 16: Other Information

References:
-Material safety data sheet issued by: la Commission de la SantÃ© et de la SÃ©curitÃ© du Travail du QuÃ©bec.
-The Sigma-Aldrich Library of Chemical Safety Data, Edition II.
-Hawley, G.G.. The Condensed Chemical Dictionary, 11e ed., New York N.Y., Van Nostrand Reinold, 1987.
LOLI, RTECS, HSDB databases.
Other MSDSs

Other Special Considerations: Not available.

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Last Updated: 11/06/2008 12:00 PM

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7.2 Reagent Volume

Although we have calculated the surface concentrations of MRS 2395 required to reach the targeted concentrations in the 4 streams (table x), the process of reagent deposition in liquid form and drying creates uncertainties in how much reagents are actually left in anhydrous form on the micro-channel. Before we conduct experimentation to study this process, the amount of MRS 2395 deposition is estimated to be 10 times the required amount in the flowing streams in this report. The computation is performed on 1X IC50 of MRS 2395 as an example. The results are tabulated in table x.

Known:

Targeted MRS concentration: IC50=3.6uM

Total Volume of Blood: 360 uL

Molecular Weight of MRS 2395: 440 g/mol

Find:

10 times the mass (mg) in this blood volume with the specified concentration.

Answer:

$$Mass\ of\ MRS_{1\times IC50} = 10 \times 3.6uM \times 360uL \times 440g/mol = 0.0057mg$$

Target concentration	Amount deposited (mg)
0XIC50, or 0 um	0
0.1XIC50, or 0.36 uM	0.00057
1XIC50, or 3.6 uM	0.0057
10XIC50, or 36 uM	0.057

The total mass of MRS 2395 deposition is the sum of MRS 2395 in 8 streams:

$$Total\ Mass_{MRS2395} = 0.00057 \times 2 + 0.0057 \times 2 + 0.057 \times 2 = 0.127\text{mg}$$

The diffusivity of ADP in water can be reasonably approximated as that of MRS 2395. Since both ADP and MRS 2395 target $P2Y_{12}$, ADP EC50 concentration can be inferred as in the same order of magnitude as MRS 2395 IC50. The same amount of 1XIC50 ADP is deposited on the channel, so the total mass of ADP is:

$$0.0057mg \times 8 = 0.0456mg$$

7.3 Channel Bifurcation Calculations

The desired width of the final eight channels is 250 µm. The height for all of the channels is 60 µm. With this desired width in mind, the effective diameter (d_{eff}) of the channel was calculated width the equation below:

$$d_{eff} = 2 * \sqrt{\frac{w*h}{\pi}} \quad (1)$$

with w = width of the channel and h = height of the channel.

Once the effective diameter is known, the length of the channel can be calculated using Equation 2:

$$l = 5 * d_{eff} \quad (2)$$

Working backwards using Murray's Law, the effective diameter of the parent channel is calculated from the effective diameter of the daughter channel.

$$r_p^3 = r_{d_1}^3 + r_{d_2}^3 + \cdots + r_{d_n}^3 = 2 * r_d^3 \quad (3)$$

Since both daughter channels have the same width, the equation can be simplified to:

$$r_p = 1.26 * r_d \quad (4)$$

After the effective diameter for the channel is calculated, the following equation is used to calculate the width of the parent channel.

$$w = \pi * \frac{\left(\frac{d_{eff}}{2}\right)^2}{h} \quad (5)$$

After these calculations have been performed, this parent channel now becomes the daughter channel for the next set of calculations and the previous steps are repeated until the first single channel is reached. The calculations were performed in Microsoft Excel and the table below shows the results,

Channel	Width (µm)	Height (µm)	Effective Diameter (µm)	Entry Length (µm)		Entry Length (mm)
Final 8 Channels	250	60	138	691	=>	0.691
4 Channels After 2nd Split	397	60	174	871	=>	0.871
2 Channels After 1st Split	630	60	219	1097	=>	1.097
1st Channel	1000	60	276	1382	=>	1.382

7.4 Dissolution of ADP and MRS 2395

Physical Constant	Value @ 37 ^{o}C
μ	$7*10^{-4}$ Pa s
ρ	992.2 kg/m^3
D_{AB}	~10^{-9} m^2/s
$C_{ADP,\ required}$	3.6 uM
x (patch length)	150 um
u (velocity)	1 cm/s
W (width chan.)	250 um

.

$$V = 0.01\ m \times 60 \times 10^{-6} m \times 250 \times 10^{-6} m = 1.5 \times 10^{-10} m^3/s$$

Substituting all the physical constants:

$$\boldsymbol{C_{ADP,s} = \left(\frac{1.506\dot{V}}{\sqrt{\frac{ux}{v}} \left(\frac{\mu}{\rho D_{AB}}\right)^{\frac{1}{3}} D_{AB} W} \right) C_{ADP} = 248\ uM}$$

All other 2 surface concentrations of ADP associated with the 2 specified ADP in-stream concentrations are computed by substituting C_{ADP} with 0.36 uM and 36 uM.

7.5 Runtime Calculations for Whole Blood Volume

Based on the widths and shear rates of the channels which model the flow of whole blood in the body, the flow rate calculated was 1 cm/s. Using this flow rate, the volumetric flow rate is calculated below for one channel.

$$\left(1\frac{cm}{s}\right)(.025\ cm)(.006\ cm) = .00015\ \frac{cm^3}{s} = \left(1.5 * 10^{-10}\ \frac{m^3}{s}\right)\left(\frac{1{,}000{,}000{,}000\ \mu L}{m^3}\right)$$
$$= .15\ \frac{\mu L}{s}$$

where velocity = $1\frac{cm}{s}$, width = .025 cm, and height = .006 cm.

The total volumetric flow rate for eight channels is then

$$\left(.15\frac{\mu L}{s}\right)(8\ channels) = 1.2\frac{\mu L}{s}$$

The amount of whole blood needed for all eight channels to flow for one minute is calculated below.

$$\left(1.2\ \frac{\mu L}{s}\right)\left(\frac{60\ s}{min}\right) = 72\frac{\mu L}{min}$$

If the channels run for 5 minutes,

$$\left(72\frac{\mu L}{min}\right)(5\ min) = 360\ \mu L$$

Extra blood is needed to verify that the collagen strip is kept covered with blood so the results are free of experimental error. It is assumed that an extra 20 seconds of blood per channel would be sufficient.

$$(20\ s)\left(1.2\frac{\mu L}{s}\right)(8\ channels) = (192\ \mu L)$$

Therefore the total amount of blood being used is

$$192\ \mu L + 360\ \mu L = 552\ \mu L$$

The waste reservoir volume has ample amount of room for this volume of blood. The volume of the waste reservoir is calculated below.

$$(.25\ cm)(3\ cm)(2\ cm) = (1.5\ cm^3)\left(\frac{1{,}000\ \mu L}{cm^3}\right) = 1{,}500\ \mu L$$

where height = .25 cm, width = 3cm, and length = 2 cm.

7.6 Image J Analysis

7.6.1 5µM MRS 2395

	High	Low	B	High	Low	Clot	C-B	(C-B)/B	Time (sec)
0001	1345	1330	1337.5	1586	1566	1576	238.5	0.178318	5
0002	1346	1328	1337	1562	1541	1551.5	214.5	0.160434	10
0003	1353	1338	1345.5	1623	1604	1613.5	268	0.199182	15
0004	1344	1331	1337.5	1589	1571	1580	242.5	0.181308	20
0005	1351	1314	1332.5	1613	1560	1586.5	254	0.190619	25
0006	1343	1308	1325.5	1599	1546	1572.5	247	0.186345	30
0007	1340	1305	1322.5	1576	1521	1548.5	226	0.170888	35
0008	1349	1248	1298.5	1580	1468	1524	225.5	0.173662	40
0009	1348	1292	1320	1634	1528	1581	261	0.197727	45
0010	1350	1260	1305	1623	1543	1583	278	0.213027	50
0011	1343	1270	1306.5	1636	1523	1579.5	273	0.208955	55
0012	1359	1247	1303	1601	1525	1563	260	0.19954	60
0013	1348	1280	1314	1622	1518	1570	256	0.194825	65
0014	1348	1289	1318.5	1622	1512	1567	248.5	0.188472	70
0015	1350	1268	1309	1617	1498	1557.5	248.5	0.18984	75
0016	1350	1258	1304	1641	1506	1573.5	269.5	0.206672	80
0017	1348	1253	1300.5	1654	1540	1597	296.5	0.227989	85
0018	1344	1227	1285.5	1620	1486	1553	267.5	0.20809	90
0019	1350	1217	1283.5	1645	1527	1586	302.5	0.235684	95
0020	1344	1223	1283.5	1629	1486	1557.5	274	0.213479	100
0021	1341	1200	1270.5	1622	1469	1545.5	275	0.21645	105
0022	1341	1251	1296	1598	1500	1549	253	0.195216	110
0023	1309	1216	1262.5	1571	1430	1500.5	238	0.188515	115
0024	1299	1205	1252	1590	1440	1515	263	0.210064	120
0025	1284	1207	1245.5	1589	1467	1528	282.5	0.226817	125
0026	1325	1230	1277.5	1620	1502	1561	283.5	0.221918	130
0027	2269	2249	2259	2755	2684	2719.5	460.5	0.203851	135
0028	2221	2142	2181.5	2663	2598	2630.5	449	0.205822	140
0029	Contaminated								
0030	1339	1317	1328	1671	1533	1602	274	0.206325	150
0031	1359	1327	1343	1674	1582	1628	285	0.212211	155
0032	1356	1330	1343	1657	1581	1619	276	0.20551	160
0033	1370	1348	1359	1704	1598	1651	292	0.214864	165
0034	1361	1328	1344.5	1664	1540	1602	257.5	0.191521	170
0035	1357	1321	1339	1671	1574	1622.5	283.5	0.211725	175
0036	1359	1323	1341	1678	1584	1631	290	0.216257	180
0037	1346	1310	1328	1702	1615	1658.5	330.5	0.24887	185
0038	1323	1286	1304.5	1670	1591	1630.5	326	0.249904	190

0039	1338	1301	1319.5	1710	1604	1657	337.5	0.255779	195
0040	1345	1301	1323	1705	1609	1657	334	0.252457	200
0041	1347	1318	1332.5	1703	1620	1661.5	329	0.246904	205
0042	1347	1310	1328.5	1708	1636	1672	343.5	0.258562	210
0043	1355	1297	1326	1730	1650	1690	364	0.27451	215
0044	1355	1317	1336	1707	1588	1647.5	311.5	0.233159	220
0045	1333	1287	1310	1729	1592	1647.5	337.5	0.257634	225
0046	1334	1297	1315.5	1673	1608	1640.5	325	0.247054	230
0047	1333	1295	1314	1689	1609	1649	335	0.254947	235
0048	1329	1292	1310.5	1698	1607	1652.5	342	0.260969	240
0049	1322	1287	1304.5	1655	1597	1626	321.5	0.246455	245
0050	1332	1301	1316.5	1737	1655	1696	379.5	0.288264	250
0051	1329	1293	1311	1675	1637	1656	345	0.263158	255
0052	1331	1298	1314.5	1666	1588	1627	312.5	0.237733	260
0053	1318	1275	1296.5	1685	1609	1647	350.5	0.270343	265
0054	1312	1275	1293.5	1678	1605	1641.5	348	0.269037	270
0055	1316	1269	1292.5	1687	1603	1645	352.5	0.272727	275
0056	1290	1263	1276.5	1669	1597	1633	356.5	0.279279	280
0057	1304	1271	1287.5	1668	1609	1638.5	351	0.272621	285
0058	1287	1259	1273	1646	1573	1609.5	336.5	0.264336	290
0059	1292	1248	1270	1649	1584	1616.5	346.5	0.272835	295
0060	1276	1254	1265	1657	1542	1599.5	334.5	0.264427	300

7.6.2 10 µM MRS 2395

	High	Low	B	High	Low	Clot	C-B	(C-B)/B	Time (sec)
0001	1756	1707	1731.5	1856	1796	1826	94.5	0.054577	5
0002	1754	1710	1732	1839	1783	1811	79	0.045612	10
0003	1753	1716	1734.5	1832	1796	1814	79.5	0.0458345	15
0004	1760	1709	1734.5	1853	1769	1811	76.5	0.0441049	20
0005	1749	1710	1729.5	1828	1778	1803	73.5	0.0424978	25
0006	1749	1700	1724.5	1829	1776	1802.5	78	0.0452305	30
0007	1721	1647	1684	1821	1743	1782	98	0.0581948	35
0008	1718	1676	1697	1797	1759	1778	81	0.0477313	40
0009	1716	1669	1692.5	1810	1750	1780	87.5	0.0516987	45
0010	1705	1650	1677.5	1801	1742	1771.5	94	0.0560358	50
0011	1719	1636	1677.5	1807	1718	1762.5	85	0.0506706	55
0012	1704	1638	1671	1793	1719	1756	85	0.0508677	60
0013	1727	1633	1680	1807	1724	1765.5	85.5	0.0508929	65
0014	1722	1632	1677	1821	1723	1772	95	0.0566488	70
0015	1720	1666	1693	1818	1730	1774	81	0.0478441	75
0016	1745	1670	1707.5	1834	1780	1807	99.5	0.0582723	80

0017	1727	1678	1702.5	1853	1779	1816	113.5	0.0666667	85
0018	1731	1684	1707.5	1840	1776	1808	100.5	0.058858	90
0019	1731	1666	1698.5	1862	1764	1813	114.5	0.0674124	95
0020	1764	1708	1736	1914	1829	1871.5	135.5	0.078053	100
0021	1790	1743	1766.5	1934	1841	1887.5	121	0.068497	105
0022	1788	1728	1758	1933	1842	1887.5	129.5	0.0736633	110
0023	1795	1672	1733.5	1954	1827	1890.5	157	0.0905682	115
0024	1788	1733	1760.5	1959	1853	1906	145.5	0.082647	120
0025	1797	1716	1756.5	1972	1872	1922	165.5	0.0942215	125
0026	1794	1755	1774.5	1961	1851	1906	131.5	0.0741054	130
0027	1805	1759	1782	1984	1903	1943.5	161.5	0.0906285	135
0028	1794	1715	1754.5	1973	1882	1927.5	173	0.0986036	140
0029	1796	1753	1774.5	1961	1889	1925	150.5	0.0848126	145
0030	1809	1753	1781	1988	1891	1939.5	158.5	0.0889949	150
0031	1808	1739	1773.5	1976	1876	1926	152.5	0.0859882	155
0032	1783	1753	1768	1966	1908	1937	169	0.0955882	160
0033	1798	1735	1766.5	1966	1903	1934.5	168	0.0951033	165
0034	1791	1690	1740.5	1987	1872	1929.5	189	0.1085895	170
0035	1787	1732	1759.5	1966	1877	1921.5	162	0.0920716	175
0036	1798	1751	1774.5	1968	1905	1936.5	162	0.0912933	180
0037	1782	1715	1748.5	1964	1817	1890.5	142	0.0812125	185
0038	1789	1740	1764.5	1976	1869	1922.5	158	0.0895438	190
0039	1800	1699	1749.5	1964	1907	1935.5	186	0.1063161	195
0040	1791	1703	1747	1976	1848	1912	165	0.0944476	200
0041	1791	1743	1767	1970	1905	1937.5	170.5	0.0964912	205
0042	1791	1753	1772	1958	1898	1928	156	0.0880361	210
0043	1775	1689	1732	1950	1888	1919	187	0.1079677	215
0044	1797	1742	1769.5	1966	1862	1914	144.5	0.0816615	220
0045	1788	1711	1749.5	1954	1887	1920.5	171	0.0977422	225
0046	1778	1655	1716.5	1953	1878	1873	156.5	0.0911739	230
0047	1784	1660	1722	1960	1882	1921	199	0.1155633	235
0048	1774	1657	1715.5	1948	1853	1900.5	185	0.1078403	240
0049	1777	1728	1752.5	1964	1882	1923	170.5	0.0972896	245
0050	1774	1711	1742.5	1952	1821	1886.5	144	0.0826399	250
0051	1764	1707	1735.5	1946	1879	1912.5	177	0.1019879	255
0052	1766	1712	1739	1943	1859	1901	162	0.093157	260
0053	1779	1664	1721.5	1960	1787	1873.5	152	0.0882951	265
0054	1760	1592	1676	1952	1848	1900	224	0.1336516	270
0055	1767	1667	1717	1952	1869	1910.5	193.5	0.1126966	275
0056	1767	1652	1709.5	1955	1892	1923.5	214	0.1251828	280
0057	1748	1697	1722.5	1946	1869	1907.5	185	0.107402	285
0058	1778	1651	1714.5	1962	1904	1933	218.5	0.1274424	290
0059	1756	1678	1717	1959	1892	1925.5	208.5	0.1214327	295

0060	1769	1704	1736.5	1962	1870	1916	179.5	0.1033688	300

7.6.3 50 µM MRS 2395

	High	Low	B	High	Low	Clot	C-B	(C-B)/B	Time (sec)
0001	1824	1768	1796	1936	1842	1889	93	0.051782	5
0002	1821	1772	1796.5	1932	1875	1903.5	107	0.05956	10
0003	1816	1789	1802.5	1909	1888	1898.5	96	0.053259	15
0004	1759	1737	1748	1883	1846	1864.5	116.5	0.066648	20
0005	1772	1731	1751.5	1914	1796	1852	100.5	0.057379	25
0006	1779	1740	1759.5	1901	1831	1866	106.5	0.060529	30
0007	1788	1757	1772.5	1897	1808	1852.5	80	0.045134	35
0008	1807	1766	1786.5	1906	1851	1878.5	92	0.051497	40
0009	1810	1753	1781.5	1914	1819	1866.5	85	0.047713	45
0010	1796	1770	1783	1918	1816	1867	84	0.047112	50
0011	1814	1768	1791	1917	1820	1868.5	77.5	0.043272	55
0012	1807	1766	1786.5	1911	1842	1876.5	90	0.050378	60
0013	1799	1767	1783	1910	1869	1889.5	106.5	0.059731	65
0014	1789	1752	1770.5	1899	1857	1878	107.5	0.060717	70
0015	1786	1741	1763.5	1895	1854	1874.5	111	0.062943	75
0016	1775	1730	1752.5	1889	1852	1870.5	118	0.067332	80
0017	1786	1730	1758	1879	1823	1851	93	0.052901	85
0018	1782	1725	1753.5	1878	1840	1859	105.5	0.060165	90
0019	1760	1720	1740	1873	1840	1856.5	116.5	0.066954	95
0020	1765	1716	1740.5	1872	1750	1811	70.5	0.040506	100
0021	1747	1705	1726	1863	1762	1812.5	86.5	0.050116	105
0022	1752	1710	1731	1856	1809	1832.5	101.5	0.058637	110
0023	1753	1696	1724.5	1846	1808	1827	102.5	0.059438	115
0024	1774	1742	1758	1851	1797	1824	66	0.037543	120
0025	1749	1688	1718.5	1871	1802	1836.5	118	0.068665	125
0026	1762	1691	1726.5	1859	1709	1784	57.5	0.033304	130
0027	1763	1628	1695.5	1828	1717	1772.5	77	0.045414	135
0028	1775	1697	1736	1849	1785	1817	81	0.046659	140
0029	1748	1694	1721	1876	1714	1795	74	0.042998	145
0030	1746	1689	1717.5	1860	1799	1829.5	112	0.065211	150
0031	1781	1686	1733.5	1878	1815	1846.5	113	0.065186	155
0032	1759	1681	1720	1840	1749	1794.5	74.5	0.043314	160
0033	1767	1712	1739.5	1845	1767	1806	66.5	0.038229	165
0034	1762	1689	1725.5	1873	1801	1837	111.5	0.064619	170
0035	1729	1674	1701.5	1842	1800	1821	119.5	0.070232	175
0036	1757	1691	1724	1862	1801	1831.5	107.5	0.062355	180
0037	1762	1685	1723.5	1841	1806	1823.5	100	0.058021	185

0038	1764	1675	1719.5	1874	1802	1838	118.5	0.068915	190
0039	1747	1650	1698.5	1871	1812	1841.5	143	0.084192	195
0040	1751	1678	1714.5	1892	1791	1841.5	127	0.074074	200
0041	1756	1656	1706	1873	1780	1826.5	120.5	0.070633	205
0042	1736	1668	1702	1855	1780	1817.5	115.5	0.067861	210
0043	1738	1638	1688	1872	1769	1820.5	132.5	0.078495	215
0044	1744	1667	1705.5	1875	1735	1805	99.5	0.058341	220
0045	1741	1699	1720	1875	1840	1857.5	137.5	0.079942	225
0046	1746	1646	1696	1881	1825	1853	157	0.092571	230
0047	1764	1723	1743.5	1885	1837	1861	117.5	0.067393	235
0048	1734	1700	1717	1871	1830	1850.5	133.5	0.077752	240
0049	1745	1692	1718.5	1888	1806	1847	128.5	0.074775	245
0050	1755	1708	1731.5	1898	1842	1870	138.5	0.079988	250
0051	1725	1674	1699.5	1898	1828	1863	163.5	0.096205	255
0052	1729	1680	1704.5	1894	1821	1857.5	153	0.089762	260
0053	1724	1685	1704.5	1898	1832	1865	160.5	0.094163	265
0054	1734	1656	1695	1903	1827	1865	170	0.100295	270
0055	1732	1653	1692.5	1936	1801	1868.5	176	0.103988	275
0056	1727	1668	1697.5	1929	1835	1882	184.5	0.108689	280
0057	1735	1668	1701.5	1954	1830	1892	190.5	0.11196	285
0058	1727	1669	1698	1952	1827	1889.5	191.5	0.11278	290
0059	1729	1686	1707.5	1962	1839	1900.5	193	0.113031	295
0060	1727	1682	1704.5	1971	1850	1910.5	206	0.120857	300

7.7 Equipment Sizing

7.7.1 Oven

Daily Chip Output (DCO) at 100% production capacity:

$$DCO = \frac{500{,}000\ Chips}{250\ days\ of\ operation} = 2000\ \frac{Chips}{day}$$

There are 2 shifts, morning shift and afternoon shift, per day.

$$Chip\ per\ shift = \frac{2000\ Chips}{2\ shifts} = 1000\ Chips$$

With the chip surface dimension of 4 cm by 6 cm, the theoretical minimum space is:

$$A_{min} = 1000 \times 4\ cm \times 6cm = 2.4\ m^2$$

Considering the fact that there are gaps between individual chips on manifold, we multiple a factor of 1.5 to obtain the actual space required from an oven.

$$A_{act} = 2.4\ m^2 \times 1.5 = 3.6\ m^2 \approx 6.2\ ft \times 6.2\ ft$$

As shown above, this is equivalent to 6.2 ft by 6.2 capacity oven. If two manifolds were to be stacked upon one another before being inserted into the oven, the space required is halved.

7.7.2 Stirred Tank

The volume of PDMS per batch is about 10 L and a factor of 1.5 is multiplied to leave extra space to buffer again overflow:

$$V = 10\ L \times 1.5 = 15\ L$$

7.7.3 Storage

Monthly Chip Output (MCO) is computed as:

$$MCO = \frac{500{,}000\ Chips}{12\ Months} = 42{,}000\ \frac{Chips}{Month}$$

One of the operating assumptions is that our inventory is shipped to clients every month, so the storage unit should be able to accommodate the volume of MCO, which is computed as:

$$V_{min\ ,MCO} = 42{,}000\ \frac{Chips}{Month} \times 6\ cm \times 4\ cm \times 1\ cm = 35\ ft^3$$

A factor of 2.0 is multiplied with $V_{min\ ,MCO}$ to consider voided volume in the storage unit:

$$V_{act\ ,MCO} = 35\ ft^3 \times 2.0 = 70\ ft^3$$

7.7.4 Profitability Input Summary

Input Summary

General Information

Process Title: High Throughput Screen of Clopidogrel Resistance
Product: Chip
Plant Site Location: Philadelphia
Site Factor: 1.00
Process Type: Discrete Operation
Operating Hours per Day: 10
Operating Days per Year: 250

Chronology

	Year	Action	Portion of Total Permanent Investment	Percentage of Total Capital Investment for Depreciation	Production Capacity (% of Design Capacity)	Product Price
Start Year	2010	Design	$93,021	0.0%		
	2011	Construction	$2,500,000	0.0%		
	2012	Construction	$2,500,000	0.0%		
	2013	Prod. Constr	$273,500	14.3%	20.0%	$100.0000
	2014	Production		24.5%	60.0%	$100.0000
	2015	Production		17.5%	100.0%	$100.0000
	2016	Production		12.5%	100.0%	$100.0000
	2017	Production		8.9%	100.0%	$100.0000
	2018	Production		8.9%	100.0%	$100.0000
End Year	2019	Production		8.9%	100.0%	$100.0000

Product Information

The Process will yield:
- **200** Unit of Chip per hour.
- **2,000** Unit of Chip per day.
- **500,000** Unit of Chip per year.

The Price per Unit of Chip is: **$ 100.0000**

Raw Materials

Raw Material	Unit of Measure	Ratio to Product	Cost of Raw Material
MRS 2395	mg	0.2540 mg per Unit of Chip	$17.00 per mg
PDMS/Curing Agent	lb	0.0240 lb per Unit of Chip	$10.00 per lb
Acetone	mL	10.00 mL per Unit of Chip	$0.0560 per mL
Collagen	mg	2.000E-05 mg per Unit of Chip	$3.500 per mg
ADP	mg	0.0912 mg per Unit of Chip	$0.1800 per mg
Glass Slide	unit	2.000 unit per Unit of Chip	$0.3125 per unit
Hydrophobic Coated Pins (20mL)	unit	1.000 unit per Unit of Chip	$0.0800 per unit
Hydrophobic Coated Pins (10mL)	unit	1.000 unit per Unit of Chip	$0.0900 per unit
Hydrophobic Coated Pin (4mL)	unit	1.000 unit per Unit of Chip	$0.0900 per unit

Equipments and Related Costs

Process Machinery	Purchase Cost	Bare Module Factor	Bare Module Cost	Purchase Year
Wisconsin Industrial Oven	$ 20,000	2	$ 40,000	2010
Biomek FX Robotic Arm	$ 15,000	1.5	$ 22,500	2010
Tankmaster UV Sterilizer	$ 1,500	1.5	$ 2,250	2010

Silverson Batch Mixer	$ 1,500	1	$ 1,500	2010
Basic Mounting Plate for Beckman I	$ 400	1.5	$ 600	2010
384 Pin Tool Head	$ 7,600	1.5	$ 11,400	2010

Storage	Purchase Cost	Bare Module Factor	Bare Module Cost	Purchase Year
McQueen Labs Refrigerator 78cf	$ 8,900	1	$ 8,900	2010

Other Equipment	Purchase Cost	Bare Module Factor	Bare Module Cost	Purchase Year
Security System			$ 2,000	2010
Approval Yr 1			$ 2,500,000	2011
Approval Yr 2			$ 2,500,000	2012
Box (free trial)			$ 273,500	2013

*Derived Bare Module Factor

Total Permanent Investment

Year: 2010

Cost of Site Preparations: **5.0% of Total Bare Module Costs**
Cost of Service Facilities: **5.0% of Total Bare Module Costs**
Allocated Costs for utility plants and related facilities: **$0**
Cost of Contingencies and Contractor Fees: **18.0% of Direct Permanent Investment**
Cost of Land: **$0**
Cost of Royalties: **$0**
Cost of Plant Start-Up: **10.0% of Total Depreciable Capital**

Year: 2011

Cost of Site Preparations: **$0**
Cost of Service Facilities: **$0**
Allocated Costs for utility plants and related facilities: **$0**
Cost of Contingencies and Contractor Fees: **$0**
Cost of Land: **$0**
Cost of Royalties: **$0**
Cost of Plant Start-Up: **$0**

Year: 2012

Cost of Site Preparations: **$0**
Cost of Service Facilities: **$0**
Allocated Costs for utility plants and related facilities: **$0**
Cost of Contingencies and Contractor Fees: **$0**
Cost of Land: **$0**
Cost of Royalties: **$0**
Cost of Plant Start-Up: **$0**

Year: 2013

Cost of Site Preparations: **$0**
Cost of Service Facilities: **$0**
Allocated Costs for utility plants and related facilities: **$0**
Cost of Contingencies and Contractor Fees: **$0**
Cost of Land: **$0**
Cost of Royalties: **$0**
Cost of Plant Start-Up: **$0**

Working Capital

Accounts Receivable	⇨	30 Days
Cash Reserves	⇨	None
Accounts Payable	⇨	None

⇨ Inventory: Days ⇨ 0.00 Unit

Utilities

Utility	Unit of Measure	Ratio to Product	Cost of Utility
Electricity	unit	1.000 unit per Unit of Chip	$0.0150 per unit

Byproducts

- no Byproducts -

Other Variable Costs

General Expenses

Selling / Transfer Expenses:	3.00% of Sales
Direct Research:	4.80% of Sales
Allocated Research:	0.50% of Sales
Administrative Expense:	2.00% of Sales
Management Incentive Compensation:	1.25% of Sales

Fixed Costs

Operations

Operators per Shift:	15 (Assuming 1 Shifts)
Direct Wages and Benefits:	$42.50 per Operator Hour
Direct Salaries and Benefits:	15.00% of Direct Wages and Benefits
Operating Supplies and Services:	6.00% of Direct Wages and Benefits
Technical Assistance to Manufacturing:	$0.00 per year, for each Operator per Shift
Control Laboratory:	$0.00 per year, for each Operator per Shift

Maintenance

Wages and Benefits:	15.00% of Total Depreciable Capital
Salaries and Benefits:	25.00% of Maintenance Wages and Benefits
Materials and Services:	100.00% of Maintenance Wages and Benefits
Maintenance Overhead:	5.00% of Maintenance Wages and Benefits

Operating Overhead

General Plant Overhead:	7.10% of Maintenance and Operations Wages and Benefits
Mechanical Department Services:	2.40% of Maintenance and Operations Wages and Benefits
Employee Relations Department:	5.90% of Maintenance and Operations Wages and Benefits
Business Services:	7.40% of Maintenance and Operations Wages and Benefits

Property Taxes and Insurance

Property Taxes and Insurance:	2.00% of Total Depreciable Capital

Straight Line Depreciation

Direct Plant:	8.00% of Total Depreciable Capital, less 1.002 times the Allocated Costs for Utility Plants and Related Facilities
Allocated Plant:	6.00% of 1.002 times the Allocated Costs for Utility Plants and Related Facilities

Other Annual Expenses

Rental Fees (Office and Laboratory Space):	**$510,000**
Licensing Fees:	**$100,000**
Miscellaneous:	**$50,000**

Depletion Allowance

Annual Depletion Allowance:	**$0.00**

7.7.5 Two Variable Sensitivity Analysis

Product Prices vs Variable Costs

Product Prices	Variable Costs												
	$7,473,400	$7,693,200	$7,913,000	$8,132,800	$8,352,600	$8,572,400	$8,792,200	$9,012,000	$9,231,800	$9,451,600	$9,671,400	$9,891,200	$10,111,000
$ 85.00	80.75%	80.35%	79.95%	79.55%	79.15%	78.74%	78.34%	77.93%	77.53%	77.12%	76.71%	76.29%	75.88%
$ 87.50	82.99%	82.59%	82.19%	81.79%	81.39%	80.99%	80.58%	80.18%	79.77%	79.36%	78.95%	78.54%	78.12%
$ 90.00	85.20%	84.80%	84.40%	84.00%	83.60%	83.19%	82.79%	82.38%	81.97%	81.56%	81.15%	80.74%	80.33%
$ 92.50	87.37%	86.97%	86.57%	86.16%	85.76%	85.36%	84.95%	84.55%	84.14%	83.73%	83.32%	82.91%	82.49%
$ 95.00	89.50%	89.10%	88.70%	88.30%	87.89%	87.49%	87.08%	86.68%	86.27%	85.86%	85.45%	85.04%	84.62%
$ 97.50	91.60%	91.20%	90.80%	90.39%	89.98%	89.59%	89.18%	88.77%	88.36%	87.95%	87.54%	87.13%	86.72%
$ 100.00	93.66%	93.26%	92.86%	92.46%	92.05%	91.65%	91.24%	90.83%	90.43%	90.02%	89.60%	89.19%	88.78%
$ 102.50	95.76%	95.30%	94.90%	94.48%	94.08%	93.68%	93.28%	92.87%	92.46%	92.05%	91.63%	91.22%	90.81%
$ 105.00	97.70%	97.30%	96.90%	96.50%	96.08%	95.69%	95.28%	94.87%	94.46%	94.05%	93.63%	93.22%	92.81%
$ 107.50	99.68%	99.28%	98.88%	98.47%	98.07%	97.66%	97.25%	96.84%	96.43%	96.02%	95.61%	95.19%	94.78%
$ 110.00	101.63%	101.23%	100.83%	100.42%	100.01%	99.61%	99.20%	98.79%	98.38%	97.96%	97.55%	97.13%	96.72%
$ 112.50	103.56%	103.15%	102.75%	102.34%	101.93%	101.53%	101.12%	100.71%	100.29%	99.88%	99.47%	99.05%	98.63%
$ 115.00	105.45%	105.05%	104.64%	104.24%	103.83%	103.42%	103.01%	102.60%	102.19%	101.77%	101.36%	100.94%	100.52%

Product Prices vs Fixed Costs

Product Prices	Fixed Costs												
	$4,077,400	$4,197,300	$4,317,200	$4,437,100	$4,557,000	$4,677,000	$4,796,900	$4,916,800	$5,036,700	$5,156,700	$5,276,600	$5,396,500	$5,516,400
$ 85.00	78.34%	78.34%	78.34%	78.34%	78.34%	78.34%	78.34%	78.34%	78.34%	78.34%	78.34%	78.34%	78.34%
$ 87.50	80.58%	80.58%	80.58%	80.58%	80.58%	80.58%	80.58%	80.58%	80.58%	80.58%	80.58%	80.58%	80.58%
$ 90.00	82.79%	82.79%	82.79%	82.79%	82.79%	82.79%	82.79%	82.79%	82.79%	82.79%	82.79%	82.79%	82.79%
$ 92.50	84.95%	84.95%	84.95%	84.95%	84.95%	84.95%	84.95%	84.95%	84.95%	84.95%	84.95%	84.95%	84.95%
$ 95.00	87.08%	87.08%	87.08%	87.08%	87.08%	87.08%	87.08%	87.08%	87.08%	87.08%	87.08%	87.08%	87.08%
$ 97.50	89.18%	89.18%	89.18%	89.18%	89.18%	89.18%	89.18%	89.18%	89.18%	89.18%	89.18%	89.18%	89.18%
$ 100.00	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%
$ 102.50	93.28%	93.28%	93.28%	93.28%	93.28%	93.28%	93.28%	93.28%	93.28%	93.28%	93.28%	93.28%	93.28%
$ 105.00	95.28%	95.28%	95.28%	95.28%	95.28%	95.28%	95.28%	95.28%	95.28%	95.28%	95.28%	95.28%	95.28%
$ 107.50	97.25%	97.25%	97.25%	97.25%	97.25%	97.25%	97.25%	97.25%	97.25%	97.25%	97.25%	97.25%	97.25%
$ 110.00	99.20%	99.20%	99.20%	99.20%	99.20%	99.20%	99.20%	99.20%	99.20%	99.20%	99.20%	99.20%	99.20%
$ 112.50	101.12%	101.12%	101.12%	101.12%	101.12%	101.12%	101.12%	101.12%	101.12%	101.12%	101.12%	101.12%	101.12%
$ 115.00	103.01%	103.01%	103.01%	103.01%	103.01%	103.01%	103.01%	103.01%	103.01%	103.01%	103.01%	103.01%	103.01%

Product Prices vs Initial Investment

Product Prices	Initial Investment (TPI)												
	$4,561,500	$4,695,700	$4,829,900	$4,964,000	$5,098,200	$5,232,400	$5,366,500	$5,500,700	$5,634,800	$5,769,000	$5,903,200	$6,037,300	$6,171,500
$ 85.00	84.16%	83.11%	82.10%	81.11%	80.16%	79.24%	78.34%	77.47%	76.62%	75.79%	74.99%	74.21%	73.44%
$ 87.50	86.56%	85.49%	84.44%	83.43%	82.45%	81.50%	80.58%	79.69%	78.81%	77.97%	77.14%	76.34%	75.55%
$ 90.00	88.93%	87.82%	86.75%	85.71%	84.71%	83.73%	82.79%	81.87%	80.97%	80.10%	79.26%	78.43%	77.63%
$ 92.50	91.25%	90.12%	89.02%	87.95%	86.92%	85.92%	84.95%	84.01%	83.09%	82.20%	81.33%	80.49%	79.67%
$ 95.00	93.54%	92.37%	91.25%	90.16%	89.10%	88.08%	87.08%	86.12%	85.18%	84.27%	83.38%	82.51%	81.67%
$ 97.50	95.79%	94.60%	93.44%	92.33%	91.24%	90.20%	89.18%	88.19%	87.23%	86.30%	85.39%	84.51%	83.64%
$ 100.00	98.01%	96.79%	95.61%	94.46%	93.36%	92.28%	91.24%	90.23%	89.25%	88.30%	87.37%	86.47%	85.59%
$ 102.50	100.19%	98.94%	97.73%	96.57%	95.43%	94.34%	93.28%	92.24%	91.24%	90.27%	89.32%	88.40%	87.50%
$ 105.00	102.34%	101.06%	99.83%	98.64%	97.48%	96.36%	95.28%	94.22%	93.20%	92.20%	91.24%	90.30%	89.38%
$ 107.50	104.46%	103.16%	101.90%	100.68%	99.50%	98.36%	97.25%	96.18%	95.13%	94.12%	93.13%	92.17%	91.23%
$ 110.00	106.55%	105.22%	103.94%	102.70%	101.49%	100.33%	99.20%	98.10%	97.03%	96.00%	94.99%	94.02%	93.06%
$ 112.50	108.62%	107.26%	105.95%	104.68%	103.46%	102.27%	101.12%	100.00%	98.91%	97.86%	96.83%	95.84%	94.87%
$ 115.00	110.65%	109.27%	107.94%	106.64%	105.39%	104.18%	103.01%	101.87%	100.77%	99.69%	98.65%	97.64%	96.65%

Variable vs. Fixed Costs

Variable Costs \ Fixed Costs	$4,077,400	$4,197,300	$4,317,200	$4,437,100	$4,557,000	$4,677,000	$4,796,900	$4,916,800	$5,036,700	$5,156,700	$5,276,600	$5,396,500	$5,516,400
$ 7,473,400	96.24%	95.81%	95.38%	94.95%	94.52%	94.09%	93.66%	93.23%	92.81%	92.38%	91.95%	91.52%	91.09%
$ 7,693,200	95.84%	95.41%	94.98%	94.55%	94.12%	93.69%	93.26%	92.83%	92.40%	91.97%	91.54%	91.12%	90.69%
$ 7,913,000	95.44%	95.01%	94.58%	94.15%	93.72%	93.29%	92.86%	92.43%	92.00%	91.57%	91.14%	90.71%	90.28%
$ 8,132,800	95.05%	94.61%	94.18%	93.75%	93.32%	92.89%	92.46%	92.03%	91.60%	91.17%	90.74%	90.31%	89.88%
$ 8,352,600	94.64%	94.21%	93.78%	93.35%	92.92%	92.49%	92.05%	91.62%	91.19%	90.76%	90.33%	89.90%	89.47%
$ 8,572,400	94.24%	93.81%	93.38%	92.95%	92.51%	92.08%	91.65%	91.22%	90.79%	90.35%	89.92%	89.49%	89.06%
$ 8,792,200	93.84%	93.41%	92.97%	92.54%	92.11%	91.68%	91.24%	90.81%	90.38%	89.95%	89.51%	89.08%	88.65%
$ 9,012,000	93.44%	93.00%	92.57%	92.14%	91.70%	91.27%	90.83%	90.40%	89.97%	89.54%	89.10%	88.67%	88.24%
$ 9,231,800	93.03%	92.60%	92.16%	91.73%	91.29%	90.86%	90.43%	89.99%	89.56%	89.12%	88.69%	88.26%	87.82%
$ 9,451,600	92.62%	92.19%	91.75%	91.32%	90.89%	90.45%	90.02%	89.58%	89.15%	88.71%	88.28%	87.84%	87.41%
$ 9,671,400	92.22%	91.78%	91.35%	90.91%	90.47%	90.04%	89.60%	89.17%	88.73%	88.30%	87.86%	87.43%	87.00%
$ 9,891,200	91.81%	91.37%	90.94%	90.50%	90.06%	89.63%	89.19%	88.76%	88.32%	87.88%	87.45%	87.01%	86.58%
$10,111,000	91.40%	90.96%	90.52%	90.09%	89.65%	89.21%	88.78%	88.34%	87.90%	87.47%	87.03%	86.60%	86.16%

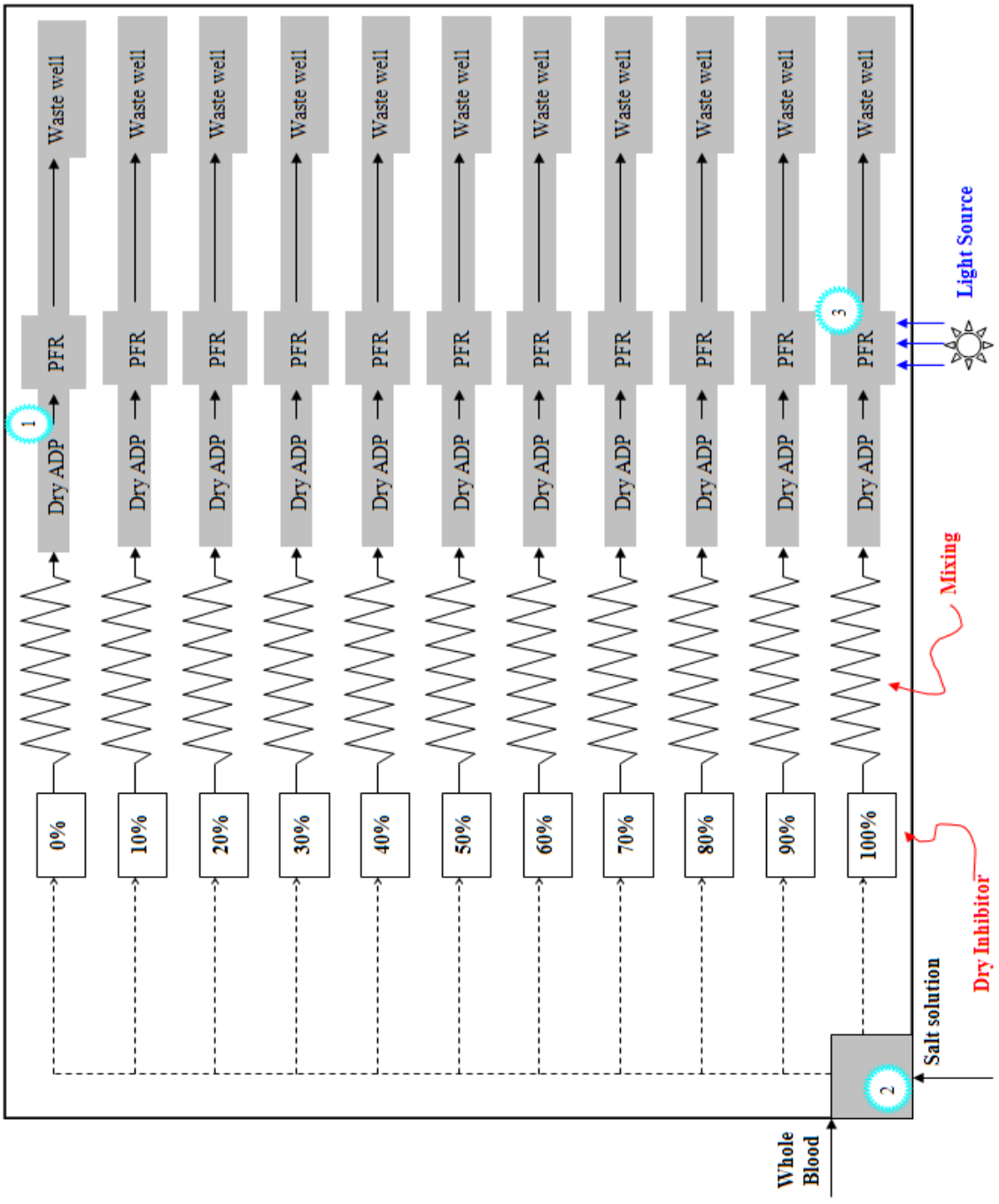
Variable Costs vs. Initial Investment

Variable Costs \ Initial Investment (TPI)	$4,561,500	$4,695,700	$4,829,900	$4,964,000	$5,098,200	$5,232,400	$5,366,500	$5,500,700	$5,634,800	$5,769,000	$5,903,200	$6,037,300	$6,171,500
$ 7,473,400	93.66%	93.66%	93.66%	93.66%	93.66%	93.66%	93.66%	93.66%	93.66%	93.66%	93.66%	93.66%	93.66%
$ 7,693,200	93.26%	93.26%	93.26%	93.26%	93.26%	93.26%	93.26%	93.26%	93.26%	93.26%	93.26%	93.26%	93.26%
$ 7,913,000	92.86%	92.86%	92.86%	92.86%	92.86%	92.86%	92.86%	92.86%	92.86%	92.86%	92.86%	92.86%	92.86%
$ 8,132,800	92.46%	92.46%	92.46%	92.46%	92.46%	92.46%	92.46%	92.46%	92.46%	92.46%	92.46%	92.46%	92.46%
$ 8,352,600	92.05%	92.05%	92.05%	92.05%	92.05%	92.05%	92.05%	92.05%	92.05%	92.05%	92.05%	92.05%	92.05%
$ 8,572,400	91.65%	91.65%	91.65%	91.65%	91.65%	91.65%	91.65%	91.65%	91.65%	91.65%	91.65%	91.65%	91.65%
$ 8,792,200	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%	91.24%
$ 9,012,000	90.83%	90.83%	90.83%	90.83%	90.83%	90.83%	90.83%	90.83%	90.83%	90.83%	90.83%	90.83%	90.83%
$ 9,231,800	90.43%	90.43%	90.43%	90.43%	90.43%	90.43%	90.43%	90.43%	90.43%	90.43%	90.43%	90.43%	90.43%
$ 9,451,600	90.02%	90.02%	90.02%	90.02%	90.02%	90.02%	90.02%	90.02%	90.02%	90.02%	90.02%	90.02%	90.02%
$ 9,671,400	89.60%	89.60%	89.60%	89.60%	89.60%	89.60%	89.60%	89.60%	89.60%	89.60%	89.60%	89.60%	89.60%
$ 9,891,200	89.19%	89.19%	89.19%	89.19%	89.19%	89.19%	89.19%	89.19%	89.19%	89.19%	89.19%	89.19%	89.19%
$10,111,000	88.78%	88.78%	88.78%	88.78%	88.78%	88.78%	88.78%	88.78%	88.78%	88.78%	88.78%	88.78%	88.78%

Fixed Costs vs Initial Investment

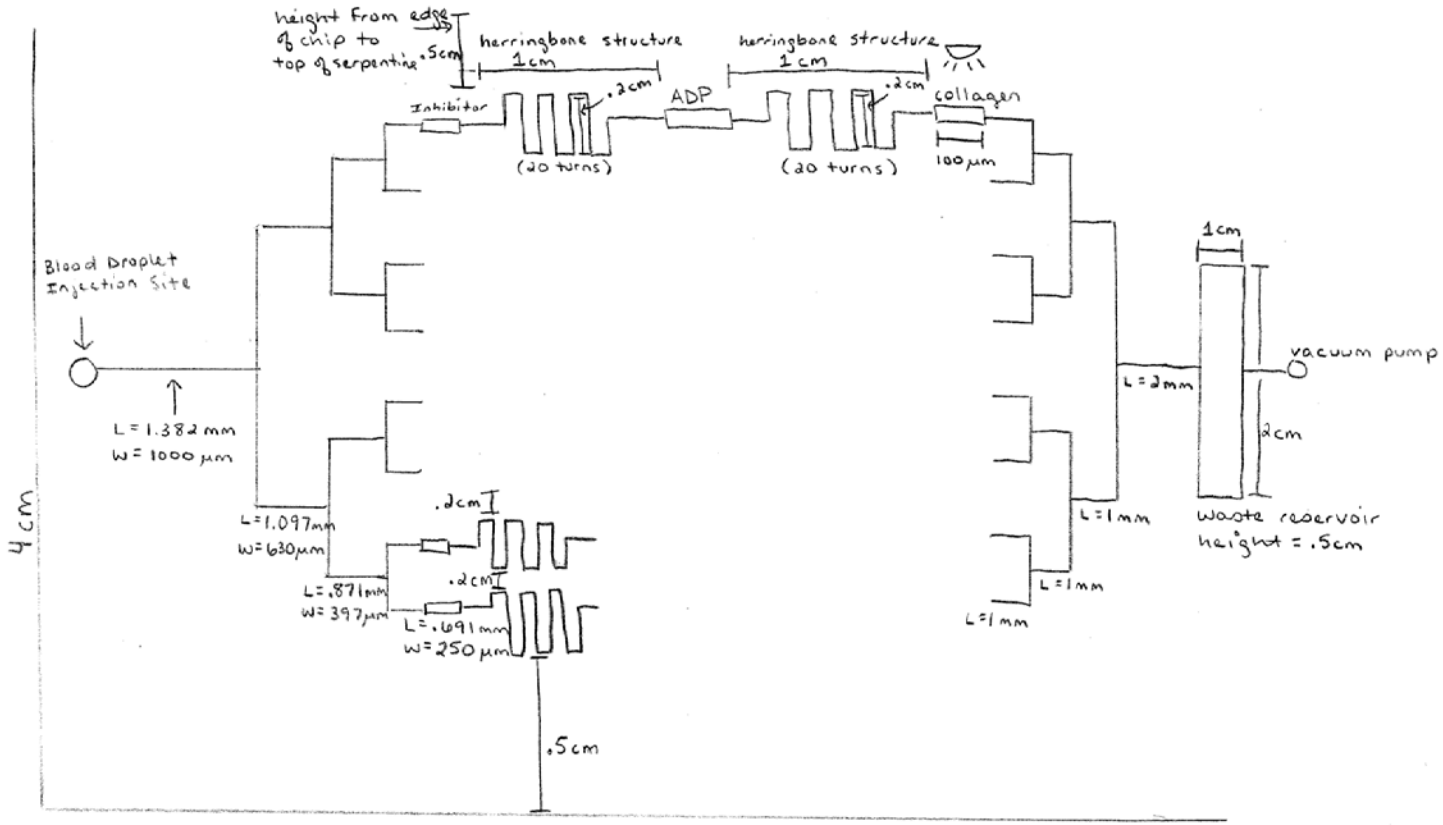
Fixed Costs \ Initial Investment (TPI)	$4,561,500	$4,695,700	$4,829,900	$4,964,000	$5,098,200	$5,232,400	$5,366,500	$5,500,700	$5,634,800	$5,769,000	$5,903,200	$6,037,300	$6,171,500
$ 4,077,400	100.87%	99.60%	98.37%	97.19%	96.04%	94.92%	93.84%	92.79%	91.77%	90.78%	89.82%	88.88%	87.97%
$ 4,197,300	100.39%	99.13%	97.91%	96.73%	95.59%	94.48%	93.41%	92.37%	91.35%	90.37%	89.41%	88.48%	87.57%
$ 4,317,200	99.92%	98.66%	97.45%	96.28%	95.14%	94.04%	92.97%	91.94%	90.93%	89.95%	89.00%	88.08%	87.18%
$ 4,437,100	99.44%	98.19%	96.99%	95.82%	94.69%	93.60%	92.54%	91.51%	90.51%	89.54%	88.59%	87.67%	86.78%
$ 4,557,000	98.96%	97.72%	96.53%	95.37%	94.25%	93.16%	92.11%	91.09%	90.09%	89.12%	88.19%	87.27%	86.38%
$ 4,677,000	98.48%	97.25%	96.07%	94.92%	93.80%	92.72%	91.68%	90.66%	89.67%	88.71%	87.78%	86.87%	85.98%
$ 4,796,900	98.01%	96.79%	95.61%	94.46%	93.36%	92.28%	91.24%	90.23%	89.25%	88.30%	87.37%	86.47%	85.59%
$ 4,916,800	97.53%	96.32%	95.14%	94.01%	92.91%	91.84%	90.81%	89.81%	88.83%	87.88%	86.96%	86.06%	85.19%
$ 5,036,700	97.05%	95.85%	94.68%	93.56%	92.46%	91.40%	90.38%	89.38%	88.41%	87.47%	86.55%	85.66%	84.79%
$ 5,156,700	96.57%	95.38%	94.22%	93.10%	92.02%	90.97%	89.95%	88.95%	87.99%	87.05%	86.14%	85.26%	84.39%
$ 5,276,600	96.10%	94.91%	93.76%	92.65%	91.57%	90.53%	89.51%	88.53%	87.57%	86.64%	85.74%	84.85%	84.00%
$ 5,396,500	95.62%	94.44%	93.30%	92.20%	91.13%	90.09%	89.08%	88.10%	87.15%	86.23%	85.33%	84.45%	83.60%
$ 5,516,400	95.14%	93.97%	92.84%	91.74%	90.68%	89.65%	88.65%	87.68%	86.73%	85.81%	84.92%	84.05%	83.20%

7.8 Prior Microfluidic Chip Designs



Microfluidic Chip Design

2/23/09

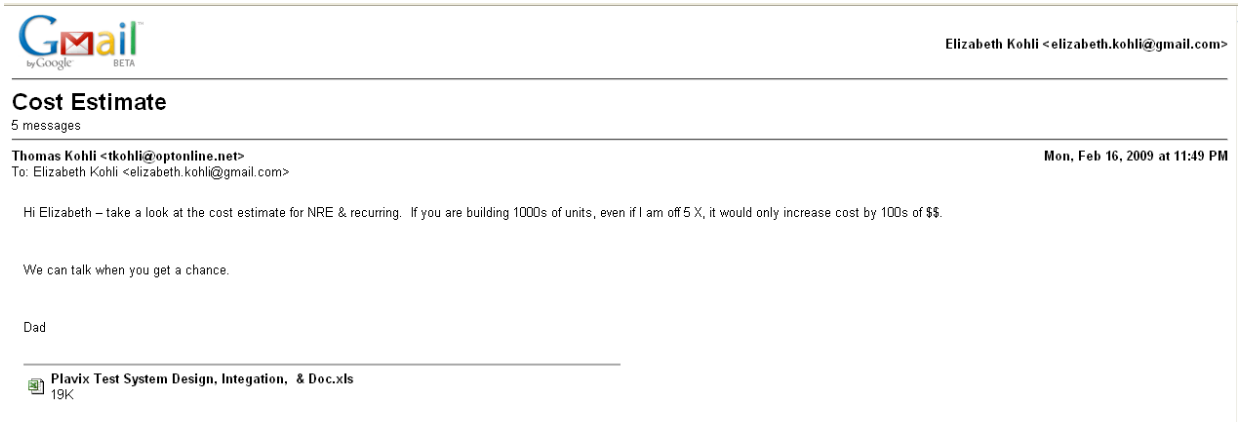


all channels: height = 60μm

chip dimensions: L = 6 cm
w = 4 cm
h = 1 cm

7.9 Consulting with Thomas Kohli

7.9.1 Recurring Costs for Box Manufacture



Gmail by Google BETA — Elizabeth Kohli <elizabeth.kohli@gmail.com>

Cost Estimate

5 messages

Thomas Kohli <tkohli@optonline.net> — **Mon, Feb 16, 2009 at 11:49 PM**
To: Elizabeth Kohli <elizabeth.kohli@gmail.com>

Hi Elizabeth – take a look at the cost estimate for NRE & recurring. If you are building 1000s of units, even if I am off 5 X, it would only increase cost by 100s of $$.

We can talk when you get a chance.

Dad

Plavix Test System Design, Integation, & Doc.xls
19K

Recurring Costs	based on a Quantity of 1			
	PC			
		Hardware	$2,000	
		Software	$2,000	assumes full version of LabVIEW; much cheaper alternative availabe but I am too tired to check now. DAD
	Component & Instrumentation Selection			
		Power Supply	$50	
		Vacuum	$100	
		LED	$25	x8
		Detector	$25	
		Interlock Switch	$25	
		Box	$100	
		Wire	$200	
		Mechanical Parts		assume all mechanical parts are machined parts. Usually a setup charge is required.
		Box Mods	$250	$125 setup & remainder is cost of item.
		Chip Hoder Brackets	$200	
		Chip Vacuum Cover	$250	
		LED Bracket	$200	
		Detector Bracket	$200	
		Box Lid with Interlock	$250	
		Silk Screens/Labeling	$250	silk screen $125 per screen
	Chip		????	
	Assembly Labor		40 hours	
	System Integration and Verification		40 hours	
	User Manual Desciption & Maintenance		40 hours	
	Certification			

7.10 E-mail Regarding Plavix Test System Diagrams

Gmail by Google BETA

Elizabeth Kohli <elizabeth.kohli@gmail.com>

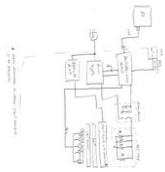
Plavix Test Sysem diagrams

3 messages

Thomas Kohli <tkohli@optonline.net> **Tue, Feb 17, 2009 at 11:06 AM**
To: Elizabeth Kohli <elizabeth.kohli@gmail.com>

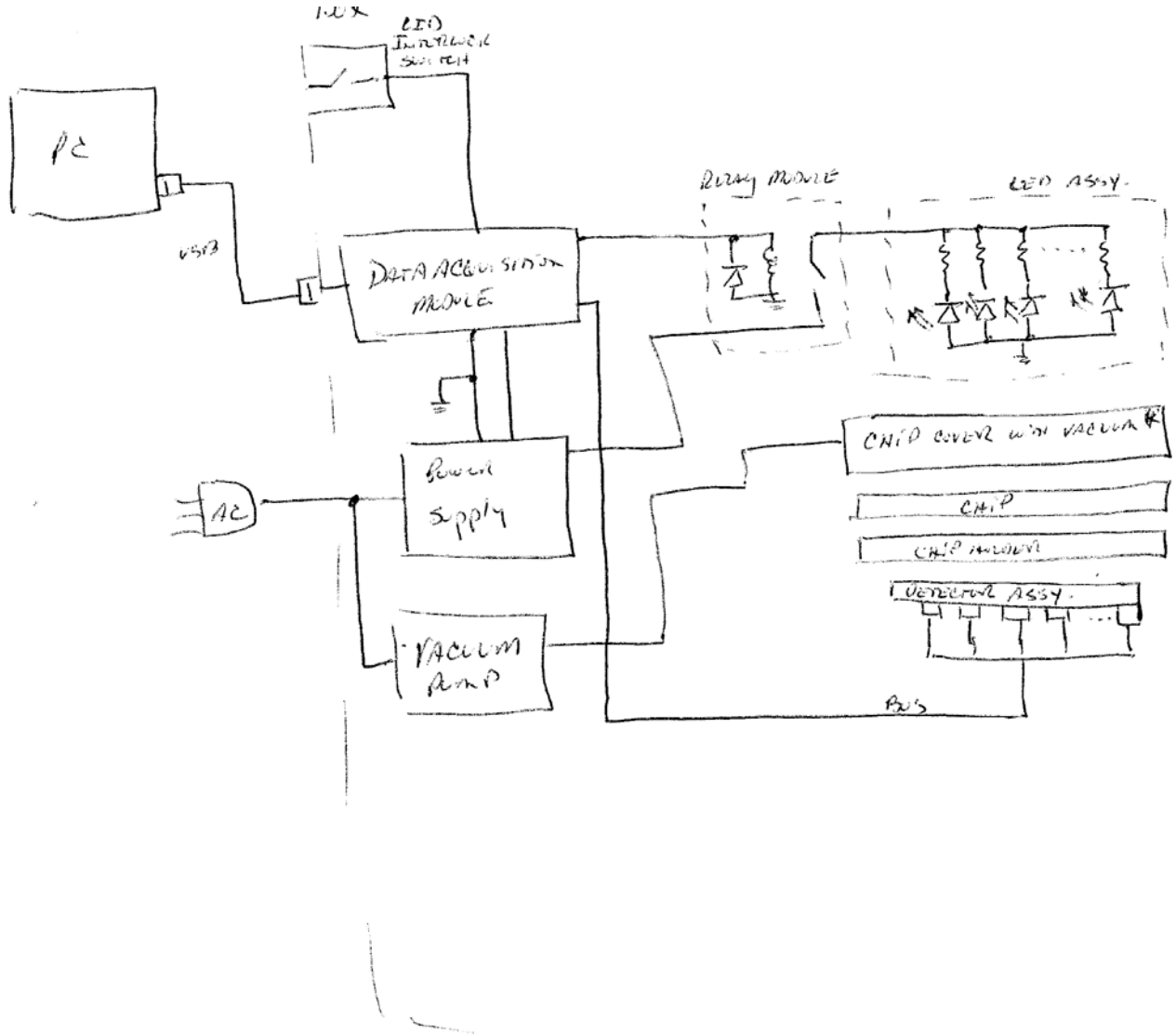
Here are two diagrams which you may need.

2 attachments



Plavix Test Block Diagram 002.jpg
568K

Plavix Test Block Diagram 003.jpg
261K



* MORE PRACTICAL TO MAKE CHIP HOLDER WITH VACUUM.

SIDE VIEW - CHAMBER INSTALLED
VACUUM COVER
CHAMBER
ASSY.
CHAMBER
ASSY.
VACUUM PUMP INSTALLED
ON VACUUM COVER
CHAMBER
CYLINDRICAL
OF
VACUUM

7.11 Velocity Profile within a Rectangular Microchannel [58]

$$u_x(y,z) = \frac{48Q}{\pi^3 hw} \frac{\sum_{n,odd}^{\infty} \frac{1}{n^3}\left[1 - \frac{\cosh(n\pi \frac{y}{h})}{\cosh(n\pi \frac{w}{2h})}\right] \sin(n\pi \frac{z}{h})}{\left[1 - \sum_{n,odd}^{\infty} \frac{192h}{n^5\pi^5 w} \tanh(n\pi \frac{w}{2h})\right]}$$

The shear rate at the wall can be found by taking the derivatives of the above expression with respect to y and z, evaluated at y=0, and z=0.

A pre-programmed excel spreadsheet made possible by Sean Maloney computed the linear velocity (u) corresponding to a average wall shear rate of 1000 s^{-1} to be 1 cm in a 250um by 60 um microchannel.

7.12 Manufacturing Equipment List

Wisconsin Oven Corporation Ltd.

SWN Series Normal Duty Walk-In Oven

Model 610-6

Specifications:

CFM X 100	Horsepower	Kilowatts-Electric	BTU's X 1,000-Gas	Cubic Feet of Chamber	Chamber Width	Chamber Width	Chamber Height
34	3	72	400	360	6'	10'	6'

Outside Width	Outside Length	Outside Height	Approximate Weight
8'	11'	9'9"	4,474 lbs.

Electro-Technic Products

High-Frequency Corona Surface Treater

Model BD-80

7.13 Communication with David Burke of Wisconsin Oven Corporation

----- Forwarded message from dj_burke@comcast.net -----
Date: Thu, 2 Apr 2009 14:51:22 -0400
From: David Burke <dj_burke@comcast.net>
Subject: Your inquiry to Wisc Oven Corp
To: paoba@seas.upenn.edu
Cc: "Hank Hubbell (WOC)" <hhubbell@wisoven.com>

Hello Paul,

Per your inquiry to Wisconsin Oven Corporation I've attempted to reach you a couple of times via phone and in order to assist you, will need to find out more about your application. Per your request, attached is a brochure of our SWN Series of batch ovens. If you refer to line 43 you will find the model SWN-610-6 you inquired about. However we don't know if you want it heated by electricity or gas and there are a lot of other questions we will need answered in order to best assist you.

The price range for this oven without any options would be in the area of $22,000.00 without any options for an electrically heated oven and around $24,000.00 for a gas fired unit. All our ovens are shipped FOB: E Troy WI, freight collect

If you would please contact me at your convenience to discuss your application and installation area in greater detail, I will be able to determine what will be required and provide you with more firm pricing.

Thank you for your interest in our thermal process related equipment, we look forward to working with you.

Regards,

David J. Burke
WOC District Sales Rep
Torrid Enterprises Inc.
978-779-0317
dj_burke@comcast.net
www.torridenterprises.com

----- End forwarded message -----

Paul A. O'Brien
University of Pennsylvania
School of Engineering and Applied Science '09
paoba@seas.upenn.edu
(845) 527-0886