Robustness and Repeatability of Interdigitated Electrodes on a Substrate Tested in an Aqueous Environment

by

Jacklyn Holmes

S.B. Mechanical Engineering
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SUBMITTED TO THE DEPARTMENT OF MECHANICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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ABSTRACT

Interdigitated electrodes are currently being used as sensing components in microfluidic lab-on-a-chip devices. The Daktari Diagnostics system uses these electrodes to measure the change in impedance of a fluid in an assay chamber. In order to improve quality assurance, a new testing method was developed and validated to characterize the sources of potential defects in the electrodes. In the new test, the electrodes are used to measure the impedance when placed in solutions of different known conductivities. The data was used to estimate the linear relationship between the inverse of the measured impedance to the solution conductivities. The repeatability tests found an average slope of 1.438×10^{-5} $^{cm}/_{characteristic\ length}$ with a standard deviation of 8.52×10^{-8} $^{cm}/_{characteristic\ length}$. It was found that the number of defective fingers or bending the electrodes significantly changes the electrode performance with a 95% confidence interval.

Thesis Supervisor: Dr. Brian W. Anthony
Title: Research Scientist

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Chapter 1: Introduction

This research focuses on the reliability of microfluidic technology that could contribute to the timely treatment of HIV patients who are hindered by the lack of resources for traditional flow cytometry to get CD4 cell counts.

1.1 HIV & AIDS

Human immunodeficiency virus (HIV) is a lentivirus that hides in the human body cells for long periods of time and attacks a key part of the immune system – the CD4 cells. CD4 cells are essential for the body in order to fight infections and diseases; however HIV can progressively destroy so many of the CD4 cells that the body loses this ability to fight. This condition is called Acquired Immunodeficiency Syndrome (AIDS). The history of HIV and AIDS is a short one. As recently as the 1970s, no one was aware of this deadly illness. [1], [2]

The HIV epidemic has become a major global public health challenge, with a total of approximately 33.4 million people living with HIV worldwide. Each year 2.6 million people become newly infected with HIV and around 1.8 million die of AIDS. The worst affected region is sub-Saharan Africa, where more than one in five adults are infected with HIV in some countries. [2], [3], [4]

1.2 Importance of Monitoring

In 2006, the United Nations Member States committed to scaling up services and interventions towards the goal of universal access to HIV prevention and antiretroviral therapy (ART) [4], [5], the main treatment for HIV. In order to achieve this objective, it is critical to escalate efforts in identifying eligible patients, effectively managing waiting lists, and closely monitoring any delays in initiation of antiretroviral therapy. [3], [6]

CD4 counting using flow cytometry is a critical component of the AIDS treatment process. It is used to identify the candidates eligible for ART, as well as monitoring of the immune system and the disease progression. [7], [8] Declining CD4-cell counts are considered to be an alarm for the progression of HIV infection. In HIV-positive people, AIDS is officially diagnosed when this count drops below 200-cells/mm^3 and antiretroviral therapy should be started. [3]

1.3 Monitoring Challenges

The standard laboratory equipment for CD4 testing is often compromised by under resourced facilities, lack of skilled health workers, deficiencies in infrastructure, and high-costs, despite the urgent need for scaling up care services in impacted areas. [6], [9] Furthermore, in decentralized hospitals in resource-limited settings, many diagnostic tests simply cannot be performed and are beyond the reach of many HIV-infected people. [10] This has resulted in CD4 tests becoming a significant barrier in the efforts to scale up HIV prevention and reaching the planned treatment target in the most affected areas. [6]

The World Health Organization (WHO) and other health organizations have urged the development of simple-to-use, affordable point-of-care CD4 cell counting system to monitor HIV-infected patients in resource-limited settings. [9], [11] Microfluidic devices have been advertised as a key candidate to accomplish point-of-care (POC) diagnostics. The technology could permit rapid tests on site, lowering the wait times for results while substantially reducing the cost to the patients. [12], [13], [14]

1.4 Use of Microfluidics

Microfluidics, and specifically lab-on-a-chip (LOC) technology, as a sub-field of MEMS/MST (Micro Electromechanical Systems/ Microsystems Technology) is an emerging technology that enables the manipulation of tiny volumes of fluid (typically in the micro to nano-liter range) in micrometric diameter channels. [12], [15] Since their introduction 15 years ago,

microfluidic devices have shown potential for providing a wide range of point-of-care (POC) applications. At Daktari Diagnostics they are developing an assay to count specific blood cells. The typical microfluidics application that is discussed in this thesis is an assay. [3], [12], [16]

Despite their potential, microfluidic POC platforms are not yet widely used outside of research laboratories. In order to market such devices and keep pace with increasing interest and demand, process optimization is essential. Their performance will also need to be thoroughly evaluated and validated in the field using clinical trials. [13], [17], [18]

1.5 Point-of-Care Development

Many medical diagnostic organizations such as Inverness, Abbott Point of Care, Daktari Diagnostics, Claros, and Diagnostics 4 All, are taking advantage of these developments and are actively involved in further advancing the development of lab-on–a-chip technology. [19], [20]

Daktari Diagnostics as a new entrant in the medical device industry has designed a simple and cost-effective point-of-care device for CD4 cell testing. The device, DAKTARI CD4, utilizes microfluidic technology for sample preparation and electrochemical sensing for measuring the CD4 cell concentration that could effectively overcome the barriers of the flow cytometry techniques. [9] DAKTARI CD4 is currently undergoing performance evaluation and optimization, and is en route to clinical trials to obtain patient results. Full scale manufacturing for the product is in development.

1.6 Development Challenges

Some of the challenges being addressed in the current manufacturing development process are: understanding the physical processes of different parts; identifying opportunities for quality control improvement; optimizing the current design; understanding the

manufacturing processes and gaining the ability to predict expected behavior; developing a system for registering the data from patients; and identifying manufacturing processes and robust materials for the full scale manufacturing stage. The performance of a component, the electrode foil, is affected in many ways by manufacturing variation and process parameters.

In this thesis, the quality control of the electrode foil has been investigated. The foil is PMMA and has an interdigitated layer of conductive material on the surface. This electrode foils is how the CD4 cell count is performed in Daktari's system.

1.7 The Masters of Engineering Capstone Project

This document is a thesis for the Masters of Engineering in Manufacturing program through Massachusetts Institute of Technology's Laboratory for Manufacturing Productivity. A team of students from the program worked on research projects with a local company. Each student focused on a different challenge. Different methods were used to understand the effects of different factors in the production of the foil and investigate opportunities to improve the design of the part. The author of this thesis, Jacklyn Holmes, focused on a new testing method and the robustness and repeatability of the electrodes. The other team members Kasra Namvari and Linda Donoghue focused on an optical system to measure the output dimensions of the electrodes [21], and the impact of design on repeatability of the electrodes and ease of quality assurance [22] respectively. The three theses in combination describe the work done by the students at Daktari Diagnostics in 2011. The majority of the first half of this document, Chapters 1-3, was written collaboratively with the other team members due to the similar nature of the projects.

1.8 Thesis Overview

Now that some background has been given about the AIDs crisis, the next chapter focuses on Daktari's product and the problem statement in Chapter 2. Chapter 3 will present the state-of-the-art for microfluidics, lab on a chip technology, CD4 cell monitoring, and the current manufacturing issues facing these technologies. Then, in Chapter 4, the methods used to approach the problem will be explained. The results and the discussion will be in Chapter 5. Chapter 6 contains the conclusion and recommendations, and Chapter 7 outlines the future work needed.

Chapter 2: Product and Project Overview

2.1 Company Background

Daktari Diagnostics is a medical diagnostic device company located in Cambridge, Massachusetts, focusing on developing diagnostic tests. The company is currently in the process of developing a CD4 cell counter for patients with HIV. The CD4 cell counter will be used in the developing world. The device is designed to be portable, robust, cost effective, and deliver results quickly as a point-of-care method. Quickly getting results will allow doctors to identify the candidates eligible for ART, and to monitor the immune system and the disease progression of the patients.

2.2 Product Description

The product currently in development at Daktari Diagnostics is a CD4 T-Cell counter that is needed for patients with HIV. The CD4 counter provides information to the caregivers about the concentration of CD4 cells in the patient's blood. This level shows how strong the patient's immune system is and can guide the caregivers as to when and how much anti-retroviral drugs (ARV) to prescribe. Measuring the CD4 cell count overtime shows how fast the disease is progressing or responding to treatment. Figure 1 shows how Daktari's system is used to get a CD4 T-Cell count. The assay process has three main stages. The stages are: (A) The blood sample flows through the assay chamber and CD4 cells stick to the antibody. (B) Other cells are washed out of the chamber. (C) CD4 cells' cell membranes are ruptured, or lysed by a high-impedance solution, and the difference in impedance is measured. [19]

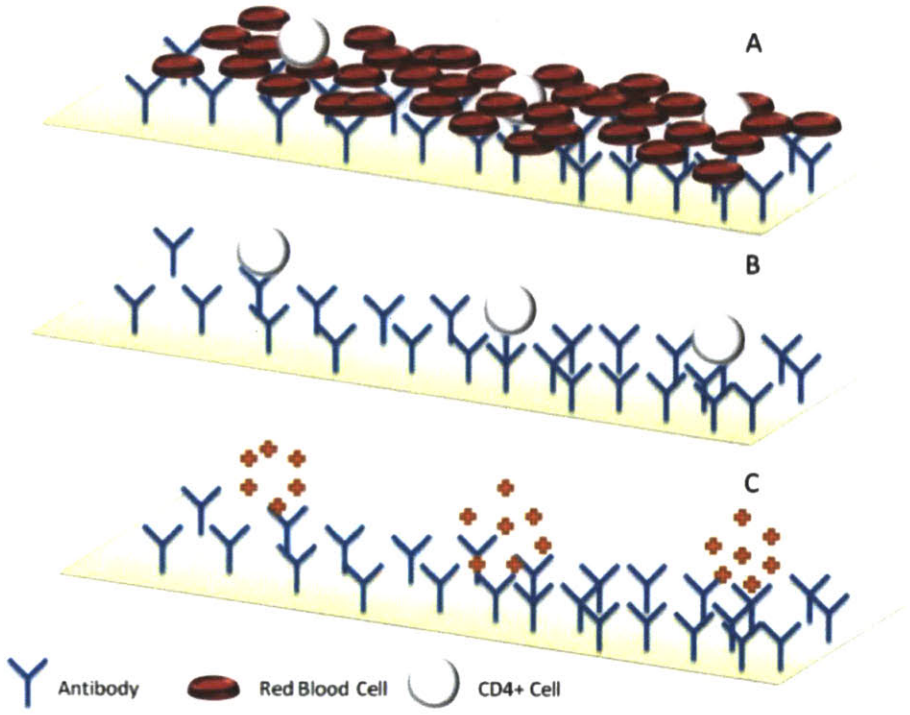

Figure 1: Assay Process Diagram [19]

This product would typically be used by a trained operator carrying the portable instrument and a supply of disposable cartridges to patients in remote locations. The device will be used where a flow cytometer is not easily accessible. The operator will prick the patient's finger with a lancet and allow the blood to flow into the sample entry port of the card. Once a sufficient amount of blood has entered the card, the operator caps the card, which seals the cartridge, and helps the patient with a Band-Aid to reduce the risk of exposure. The capped card then goes into the instrument, and the test would start. Solenoids in the instrument drive the fluid reagents, which are stored in blisters on the card, out of the blisters in a controlled manner. Actuation of valves guides the sample through an assay chamber. Antibodies that were deposited to the electrode foil capture the CD4 cells. The captured cells' cell membranes are ruptured, or lysed by a high-impedance solution. The contents of the cells reduce the impedance of the solution. This reduction of impedance is then used to measure the concentration of CD4 cells in the blood sample; subsequently the concentration is displayed on the instrument's LCD display.

2.2.1 The Instrument

The battery-powered instrument is designed for portability. It contains the actuators for driving the reagents and operating the valves. The instrument connects to the electrode in the cartridge to read the impedance measurements in the assay chamber. The measurements are used to determine the CD4 cell count in the sample, and the rest of the electronics needed to display the results and drive the actuators are contained in the instrument. The instrument is also the user interface while a test is being performed. Figure 2 shows how the disposable cartridge will go into the unit. Inserting the cartridge is similar to how a cassette goes into a cassette player. All of the tasks are met by the subassemblies listed below.

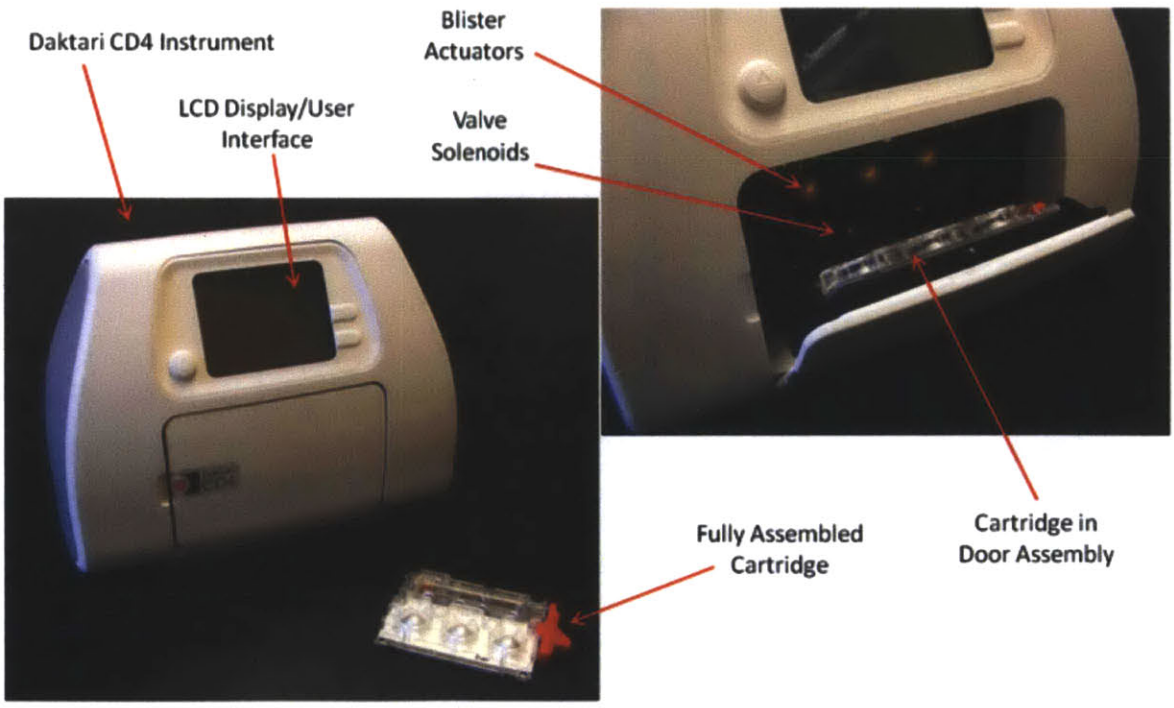

Figure 2: Daktari Instrument [22]

1. Frame - the structural element of the instrument. All of the other subassemblies are located using the frame.
2. Door Subassembly - locates the cartridge, punctures a vent hole and ensures no deformation of the card.

3. Actuator Subassembly – holds the actuators perpendicular to the frame.
4. Solenoid Subassembly – holds the valve actuators perpendicular to the frame.
5. Outer Casing – protects the internal components from impact and debris and also provides an aesthetic appeal.

2.2.2 The Cartridge

The cartridge is the consumable for the test. The cartridge is a microfluidic device with reagents and the sensing mechanism to measure the amount of CD4 cells in a sample of blood. Figure 3 shows a recent iteration of the design. Each cartridge contains the following 7 parts:

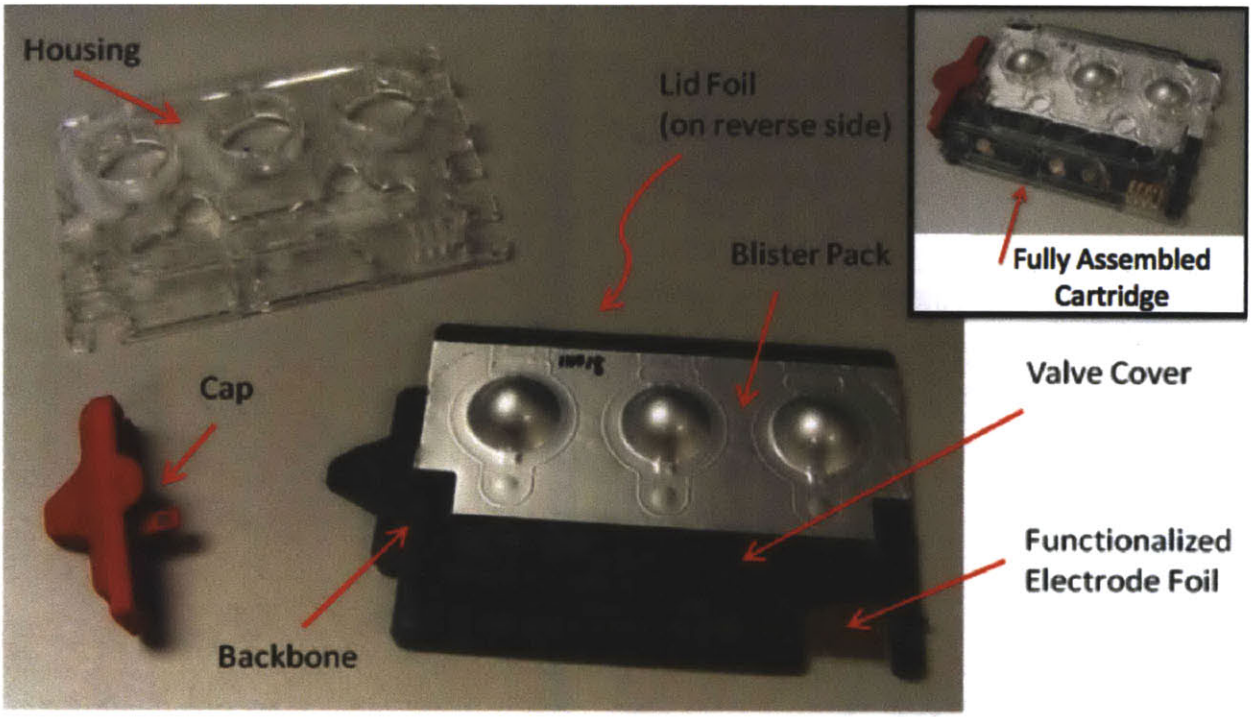

Figure 3: Daktari Cartridge with all Seven Components Labeled [22]

1. Backbone – an injection molded PMMA (polymethyl methacrylate) card with microfluidic channels.
2. Lid foil - a transparent PMMA sheet that is laser welded to one side of the backbone to seal the microfluidic channels on the backbone.

3. Functionalized electrode foil – a PMMA foil that covers the 'assay chamber' where the CD4 cell count is performed. This foil has an electrode layer on it. It is then coated with antibody solution, which is used to trap the desired CD4 cells.
4. Blister pack – the three semispherical objects in Figure 3 contain the three liquid reagents that perform tasks as they flow through the system.
5. Valve cover- a layer of polymer used to create a seal on the valves that are used to direct flow through the system.
6. Housing - an injection molded PMMA element that protects the blister pack and functionalized foil.
7. Cap – a polymer component that seals the blood entry port after the blood is sampled and also closes vents that were necessary to allow capillary flow of blood into the card.

2.3 Problem Statement

In 2011, Daktari Diagnostics was in the process of industrializing the manufacturing processes for production of the cartridges for the clinical trials and preparing for product commercialization. This transition requires focus on the production processes and capabilities in order to efficiently produce parts and maintain the quality required for the final product.

In the course of this transition, Daktari has encountered several problematic areas where the manufacturing processes were unable to deliver parts as needed for the development process. These setbacks were due in combination to current process limitations as well as assay limitations, whereby making the part more easily manufacturable would affect the operation of the final product. In many cases, these issues, such as molding the microfluidic backbone, were solved by minor design alterations or further development and optimization of the existing processes. Others required significant research and development to reach by new design features, new materials, or the development of a new process.

The overlying theme of all challenges in the scale-up process is quality control and mitigation of variation for the final product and assay results. This theme was adopted as the main focus of

the projects at Daktari, with the goal of identifying sources of variation, determining allowable tolerances to this variability, and offering solutions to monitor and control the manufacturing quality.

In 2010, Linares [24] and Selvakumar [19] performed a survey that highlighted the most critical manufacturing challenges facing Daktari at the time. The survey included potential failures of individual parts as well as part interactions that were critical to operation. The next sections will discuss the progress made in the past year, outstanding challenges, and some additional considerations that have influenced the focus of this project. Based on these observations, the main focus of this project is the robustness and repeatability of the electrode foils.

2.3.1 Manufacturing Challenges

In this section a range of manufacturing challenges for the components of the cartridge as well as the interactions of these components with the instrument are described.

a. ***Blister Pack Production and Instrument Interaction***

Previous research on the formation of the reagent blister packs and modeling the flow behavior in order to determine the effects of formed geometries and instrument alignment on both the flow characteristics and assay performance. [19], [24] Additionally, instrument and cartridge interactions at the valves and electrode pads were also analyzed. This work provided extensive information on component behavior during product operation. Conclusions from this work have led to the optimization of blister geometry, as well as continued work on valve design and flow analysis.

Recent challenges in regards to blisters and fluid flow are valve leakage and an occurrence of post flow. Valve leakage is one potential cause for unexplained fluid behavior, and prompted a redesign of the valve seat geometry, a change of valve material from blister foil to a polymer, and experimentation on the instrument actuator tips. These design changes successfully reduced the risk in the operation of these parts. Further design modifications will continue if the problem resurfaces.

Post flow is a phenomenon where fluid flow continues, sometimes for minutes, after stopping actuation of the blisters. This occurrence appeared to be a response in which stored energy caused fluid to continue to flow into the system after the forced actuation. In the end, a minor redesign to the blister system eliminated this problem.

b. Electrode Foil Production:

As described in Section 2.2, the electrode foil consists of an interdigitated electrode pattern on a PMMA substrate. The electrode is critical to the operation of the Daktari CD4 system, which relies on the electrical readings from the electrode to determine the cell count. The nature of the impedance reading makes it sensitive to minor variations in the electrode, which has previously been a fragile part. Daktari is currently developing and validating a new manufacturing process to produce robust parts. To assure accurate assay results, it is critical to understand the production variability and to ensure repeatability in electrode manufacturing.

The previous method of electrode production employed Chemical Vapor Deposition (CVD) to sputter gold over the entire surface of the PMMA substrate, followed by laser ablation to strip away unnecessary gold, leaving the electrode pattern behind. The gold electrodes were fragile, required delicate handling, and created risk for an assay that relies on the exact finger configuration and continuity to produce repeatable results. In addition to the risk of damaging the electrodes post-production, the ablation process itself introduced variability. During ablation, gold particulates redeposit onto the substrate, texturing the surface and making the foil more difficult to weld to the backbone.

Daktari Diagnostics has recently developed a new process for the production of the electrode foils. The new process is currently being patented and the details will not be discussed in this thesis. These new parts are far more robust as well as faster and less expensive to produce. While initial observations indicate the viability of these new parts in the product, validation is required before the gold electrodes can be abandoned. To get to the point of abandoning the gold electrodes, Daktari must eliminate or understand how to control any risks associated with the

new product. The large majority of these risks relate to the quality of the parts produced and the variability that may affect the performance of the CD4 assay.

2.3.2 Project Objectives

The main goals the team has developed are:

- Verify the performance of the new electrodes
- Investigate sensitivity to shipping, handling, and defects
- Quantify the variability in the new electrode production
- Understand the process parameters used to produce the electrodes
- Propose design changes to improve the performance of the new electrodes.
- Identify quality control methods for large-scale production

2.3.3 Individual Focus

In this project, the author was tasked with understanding the robustness and repeatability of the new electrodes. The project had three main goals. The first goal was to develop and characterize a testing method to quickly measure the expected performance of an electrode and find the relationship between the testing methods. The second goal was to prove that the electrodes are in fact robust and can maintain performance after shipping and handling. The final goal was to evaluate the impact of defects on electrode performance.

Chapter 3: Background Research

This chapter introduces the state of the art for microelecto-mechanical systems. From there, the uses and advantages of microfluidics will be discussed. More information will be given on the components and structures necessary in microfluidics specifically for the use in Lab-on-a-Chip technologies. Also in this chapter, the reasons for CD4 counting and the current methods for performing the count are presented. Cell Lysate Impedance Spectroscopy is an alternative to the current methods of counting, with unique manufacturing challenges. Finally a brief review of statistical process control will introduce the methods used in this thesis to test the electrodes and verify results.

3.1 MEMS

A major drive in modern technology over the past several decades has been miniaturization. The field of microelecto-mechanical systems (MEMS) was founded three decades ago when scientists began making leaps forward in miniaturization. MEMS technologies include many variations of electromechanical devices in the hundreds of micrometer to sub-micrometer scale. Devices range from gears to full electrostatic motors and micro-engines. These small-scale systems are much like integrated circuits, with their ability to offer integrated operations and functionalities on a single chip. Commonly used as sensors, MEMS can be produced and incorporated in product designs to handle detection, analysis, and signal processing in a small and repeatable package.

As a research tool, MEMS are instrumental in taking measurements and observations that were previously impossible due to difficulty in operating at that scale, such as quantum behavior and sub-molecular phenomena. MEMS are also beneficial to modeling macro behavior for miniaturization purposes as well as resource availability, process control, repeatability of experiments, and degree of observational details. Properly mimicking macro behavior and responses can be a challenge because of the inflation of effects that are

negligible or relatively small at the macro level but significant in the micro range, such as adhesion.

3.2 Microfluidics

A specific focus spun off from MEMS research is microfluidics, the study of fluid flows through micro-scale structures. These systems are utilized for many purposes including micropumps, microvalves, and micromixers. Microfluidic devices can function independently or form an integrated system of channels, mixing chambers, nozzles, etcetera, which perform entire processes. This full integration capability, carried over from MEMS development, lends many of the same advantages specified to MEMS. These and other advantages of microfluidics, as highlighted by Land [25], are as follows:

- Efficient use of reagents, minimizing resources and expenses
- Flexible and modular devices which can be combined for scaling
- Faster analysis, with potential for nearly real-time results
- Tighter control of processes through precision with small volumes (especially in use of droplets)
- Low cost of production per unit

Microfluidics are especially attractive for system because the elimination of moving parts simplifies production by integrating many elements. As also stated for the general realm of MEMS technology, one of the challenges is accounting for the different dynamics of the small scale. Even moving a fluid through a simple channel must be reconsidered at this scale. Adhesion forces, fluid particle size, boundary conditions, and other phenomenon must be examined to determine the importance of each. [25], [26]

3.2.1 Components of Microfluidics

As previously stated, microfluidic devices perform a range of functions, often in combination with each other. The main components which form these building blocks can be generalized into three main categories, as described by Tabeling [27]: fluidic interconnects, control elements, and fluid injection.

Fluidic Interconnects: Interconnects serve as connectors to microfluidic channels from other microfluidic channels, external input, or fluid injection components.

Control Elements: These components, such as pumps and valves, allow the flow of fluid to be controlled and regulated as desired.

Fluid Injection: Injection components, such as microneedles and capillary channels, facilitate the sample preparation and introduction into the microfluidic system.

The components listed above describe the construction of and basic fluid flow within microfluidics. Many more features and tools can be employed for operation, fluid manipulation, and specific fluid processes. The wide array of microfluidics can include microvalves, micropumps, microneedles, and microseparators. [28]

Each of these can be described as multi-purpose tools, bridging the general categories above, and are available for a number of applications through integration in microfluidic platforms.

3.2.2 Microfluidic Device Structure

Microfluidic devices are composed of different layers, each performing a specific function. The typical arrangement of a microfluidic device is shown in Figure 4; it is comprised of a central layer or a "backbone", external layers, and additional components for flow control and sensing applications.

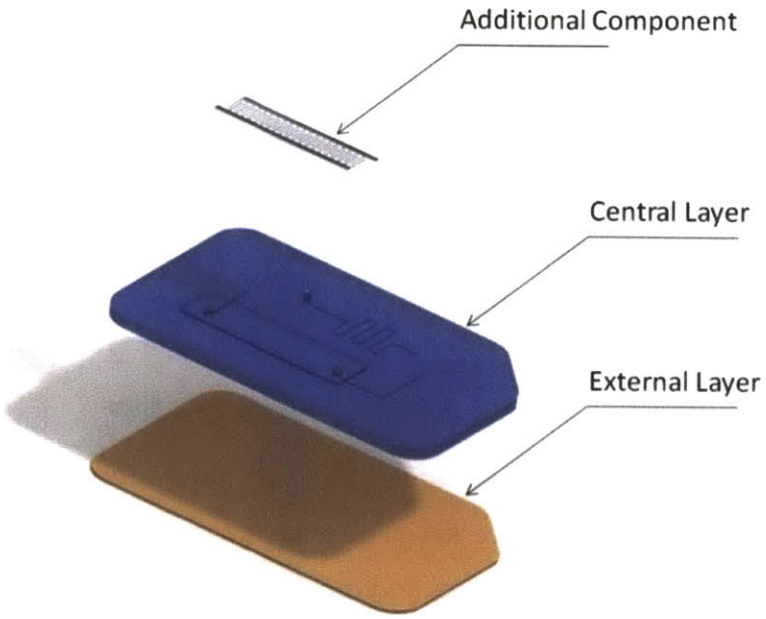

Figure 4: Microfluidic Layered Architecture [24]

Central Layer

The central layer, or backbone, is the core of microfluidic devices. The backbone contains all the microfluicic channels, valves, vents and waste channels and all other elements are mounted to it. This central layer can have varying levels of complexity with the channels depending on the application.

The backbone developed at Daktari contains all of the features mentioned above and is manufactured as a plastic injection molded component using PMMA (polymethyl methacrylate). Other components of the Daktari CD4 cartridge, including the electrode foil and the blister packs, are mounted to the backbone.

External Layer

The external layer acts as a cover for the central layer and seals the microfluidic channels. At Daktari, this layer is a transparent PMMA film laser welded to the backbone. This film seals the channels with no additional functionality. The electrode film performs the same function over the assay chamber on the reverse side of the cartridge. The PMMA substrate

is welded around the channel, serving as the external layer and providing the seal, while also performing the critical electric sensing function of the assay.

Additional components

Depending on the application, additional components are added to the external layers. These components typically perform fluid flow control or sensing.

Fluid flow control mechanisms are features on the central layer, such as valves, or external components for directing or implementing fluid flow within the central layer. At Daktari, the blister pack containing the three reagents is an additional component that allows for the delivery of reagents to the central layer and drives the reagents through the system.

Sensing components are used for measuring changes in different properties such as temperature, pressure and electrical properties. Daktari's electrode foil is utilized to measure the impedance change in the assay chamber.

3.3 Lab-on-a-Chip Technology

A study by Korb [18] sought to identify potential applications for microfluidics; many of these applications fell into the biochemistry and other related fields. The ability to combine microfluidic processes with other MEMS technology led to the development of lab-on-a-chip devices, which complete portions of or full chemical and biochemical processes. These processes include drug delivery systems, assays, genomics, cytology, and surface patterning, among many others.

Typical laboratory operations will include individual stages for sample preparation, pre-treatment, separation, and reactions, in addition to measurements, observations, and the interpretation of results. With careful design, complete lab-on-a-chip devices can incorporate all of these procedures and produce results from the input of a small raw sample. In addition to the simplicity of the test, the rate can be much faster due in combination to the small samples requiring reactions and the elimination of preparation

and material handling steps. Rates can reduce from hours or days of processing to minutes or hours respectively. [25]

The Daktari CD4+ system utilizes microfluidic channels in the cartridge to flow blood and reagents that are controlled by actuators and solenoids in the instrument. The design of the product takes a fixed volume of blood, collects the desired cells, and processes the sample to determine and report on the concentration of CD4 cells. This use of microfluidics takes advantage of a small sample size, minimal sample preparation, and the processing and delivery of results by a single device. This was achievable by the integrated system of fluid introduction, channels and valves, and processing of the sample and reagents. This lab-on-a-chip process supplies a result in a few minutes and does so with a portable instrument, eliminating the need for elaborate laboratory equipment and training.

3.4 CD4 Testing

The CD4 concentrations allow doctors to assess the relative health of a person with Human Immunodeficiency Virus (HIV) or Autoimmune Deficiency Syndrome (AIDS). The cell count in a patient's blood sample is used to determine when the patient should begin a treatment regime of antiretroviral therapy (ART). Treatment is initiated when the cell count drops below a certain level, which varies depending on available resources. WHO standards call for treatment when CD4 levels fall below 350 cells/μL, although this is often reduced to 200 cells/μL (the official level at which a patient is declared to have AIDS) in resource limited regions such as those of Daktari's focus. During treatment, additional CD4+ tests are conducted to monitor the effectiveness of treatment and the overall health of the patient in terms of their immune system. [29]

Traditional testing for CD4 cell concentrations is performed through flow cytometry. This process involves marking CD4 cells from a blood sample with a fluorescent marker, flowing cells past an excited light source, and utilizing a photomultiplier to detect the changes in wavelength as each cell passes. This allows the absolute CD4 cell count to be determined for the given sample. The equipment involved is very large, complex, and the test is time

consuming, and must be performed by trained personnel at stationary laboratories. Additionally, they require larger samples and sample preparation prior to CD4 counting. This preparation and testing process can take 18-24 hours to complete, not including other delays. Flow cytometry processes are often in high demand, resulting in long lead times before receiving results. [29]

3.4.1 Cell Lysate Impedance Spectroscopy

Flow cytometry is a well-known cell counting procedure but the machine can be difficult to maintain and few machines are available in the developing world. The Daktari CD4 system takes a different approach to CD4 testing, using cell lysate impedance spectroscopy. In this process, antibodies in the assay channel retain the CD4 cells as blood is flowed through the microfluidic channels. Reagents wash out the other cells and ions in the blood and lyse the remaining white blood cells, causing a drop in the measured electrical impedance in the channel. The assay, which determines the concentration of CD4 cells, is completed with an electrochemical sensor, which takes the change in electrical impedance after lysing to determine the concentration in a small sample of blood. The magnitude of the impedance drop has a linear relationship to the number of cells lysed, allowing the number of cells to be determined by converting the change in impedance to the number of cells based on the relationship. In testing, these results compare closely to the traditional flow cytometry method.

3.5 Manufacturing

There are a multitude of ways to make microfluidic devices and even more new techniques being developed with a wide array of materials and properties. The manufacturing technique chosen by any microfluidic designer greatly depends on the material and tolerances required in the design.

3.5.1 Backbone Manufacturing Options

Daktari Diagnostics chose to use polymethyl methacrylate (PMMA) for the microfluidic backbone because it interacts well with the fluids and chemical components in the assay. This material choice was carried through with other plastic components in the card in order to maintain compatibility and consistency in material properties.

Polymers, such as PMMA, can be processed in either serial or parallel processes. Serial processes are less desirable due to the response of the polymer to intense localized energy, such as those that occur in milling processes. The long chains that make the polymer can reorient when energized and crystallize in an undesirable form. Therefore, most processing of polymers for microfluidic applications is a parallel process where the entire surface is patterned at once with the use of a mold. Molding applications available include injection molding, micro-casting, and micro-forging. [18]

The polymer is formed around the mold to get the desired shape. This molding is generally a standard process; however, the processes used to make the mold are diverse. Many techniques have been adapted from other industries such as semiconductor manufacturing. [18]

One such technique is to apply a photoresist to a substrate and cure the negative of the microfluidic pattern. This mold is relatively quick to manufacture, but cannot produce a large amount of parts. Similarly, using a photoresist and etching process to make the mold out of silicon can create a more robust mold but requires more processing steps.

Electroplating and Electro-Discharge Machining (EDM) are additional options. Electroplating is commonly used in conjunction with physical vapor deposition (PVD). Physical Vapor Deposition is used to create the initial layer and the electroplating grows on top to create the mold. Electro-Discharge Machining (EDM) electrically erodes away unwanted material from the mold. [18]

Daktari uses traditional machining methods to create molds for the plastic injection molding of the backbone, cap, and housing. The mold is expensive and not infinitely flexible to design changes, but each mold can create many parts and does not vary much from part-to-part. This process was also chosen for the speed that injection molding could produce parts in production. Daktari's partner, who produces the injection-molded parts, can meet the necessary dimensions and tolerances with this process.

3.5.2 Electrode Foil Manufacturing Options

The electrode foil performs multiple duties in the Daktari cartridge and requires several steps to produce a complete part. A complete electrode consists of a PMMA substrate, electrode-sensing layer, and antibody solution with a protective layer. The process to produce complete and functionalized electrodes starts with a sheet of extruded PMMA. This sheet will first have the electrode layer put on the surface before the antibody and protective coating are applied onto the surface by spotting, which is the deposition of small drops of solution.

There are two methods Daktari is using to apply the electrode to the substrate. The first method is to use PVD to sputter coat the PMMA entirely with gold, followed by a laser ablation of the excess gold from the surface. Gold was chosen due to its conductivity properties and resistance to corrosion. The ablation process is done by raster (a serial process) or excimer (a parallel process) laser methods, each of which presents its own challenges. The rastering laser textures the surface while the excimer process causes some amount of gold to redeposit on the surface. Both side effects complicate the laser welding process and affect the properties of the electrode. An additional challenge with the gold electrodes is the poor adhesion between the gold and PMMA substrate. The poor adhesion causes the electrodes to be fragile and susceptible to damage, resulting in broken electrical connections and variability in the measurement of the assay.

The second method was developed at Daktari Diagnostics and the electrodes produced are the focus of the 2011 thesis projects. The electrodes fashioned by this process are much

preferred from a manufacturing and durability standpoint. They are faster to produce, configurable for flexible design changes, and are more resistant to physical damage than the gold electrodes.

Once the conductive electrode layer is complete, the antibody and a protective coating are applied to the PMMA with the electrode by spotting. The spotter deposits small drops of antibody solution to the surface of the electrode in the area shown in Figure 5. The spotter then deposits a layer over the antibody as a protective coating. There is a spotting machine located at Daktari's facility in Cambridge Massachusetts and one at Daktari's partner in Germany. The exact pattern of the antibody will depend on the characteristics of the final electrode process and pattern.

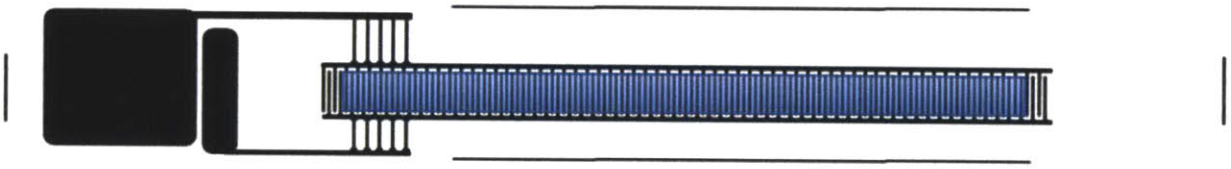

Figure 5: Electrode with Antibody Range in Blue

It is the goal of these thesis projects to validate the sensing performance of these electrodes and justify the commitment of future electrode manufacturing with the new process.

3.6 Statistical Quality Control

This section outlines the statistical tools used in the analysis of the data collected for this thesis. The two following topics were the most widely used statistical tools.

3.6.1 Student's t-test

Some inferences needed to be made about the process quality. For this project, the author assumed that results were normally distributed. However, the mean and standard deviation for the tests done in this thesis were not known. Because of that, the Student's t-distribution was used. The Student's t-test, or t-test for short, was used to assess the

statistical significance between the means of two sample populations. A confidence interval of 95% was used for the experiments. [30]

3.6.2 Design of Experiments (DoE)

Designed experiments methodology helps the user find results through a systematic way of changing the inputs. Replicates are used to deal with random behavior. The experimental objectives can included identifying which variable are most influential on the output, meet a required output, or reduce noise in the output. These experiments are used to actively make changes to the input and then observe the resulting change in the output. [30]

To analyze the data, the software called JMP was used. [31] The software was designed to do statistical analysis. The author used this software to also help design the DoE. This software was used to interpret this data, performs residual analysis, and verity the validity of resulting models. The JMP software was extensively used to create the analysis of variance (ANOVA).

Chapter 4: Methodology

As mentioned in the previous section, Daktari Diagnostics had developed a new manufacturing process for the production of the electrode foils. The new process was being patented. Because of the patent process, no information about the process could be released in this thesis. Although this process seemed to be producing viable parts, further validation was required to ensure the quality and the consistency of produced parts. This thesis investigated a new electrode testing method, and the electrodes' robustness to shipping, handling and production defects. The projects undertaken by the team were determined after investigation of the physics of the electrodes. A brief review of the physics of interdigitated electrodes is given before the details of the testing.

4.1 Electrode Review

The electrode foil is part of the cartridge that is used to measure the number of captured CD4 cells. The pattern used for the testing, which is not to scale, is shown in Figure 6. Figure 7 shows a subassembly of an electrode and backbone. The electrode foil also performs two structural tasks when it is welded to the backbone. The foil forms the ceiling of the 'assay chamber' where the CD4 cell count is performed and is spotted with antibody solution, which is used to capture the CD4 cells.

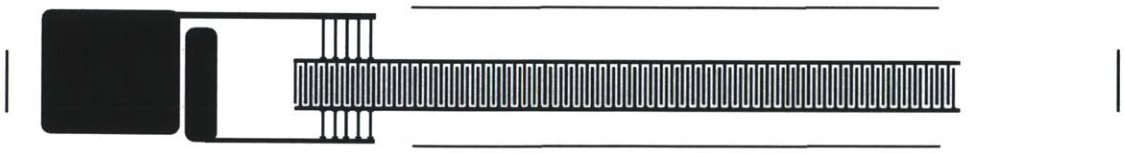

Figure 6: Electrode Foil Pattern

Figure 7: Electrode and Card Subassembly [21]

During operation, the electrode foil measures the electrical impedance within the assay chamber, both before and after lysis, or bursting, of the captured CD4 cells. The measured electrical impedance drops due to the release of ions from the cells and is directly proportional to the number of CD4 cells from the sample. Pads at the end of the electrode establish contacts with the electric connector pins in the instrument. The instrument takes the measured impedance change and converts it to a cell count to display to the user. The cell count is determined based on a predicted linear relationship between the impedance drop and cell count.

The quality of the new electrodes and the constancy in the dimensions of the fingers and the side rails are vital in the performance of the electrode foils. Impedance measurements are sensitive to the sensing area of the electrode. This sensing region is the space between opposing electrode rails. An interdigitated electrode, such as Daktari's, increases the sensing region for a given area by utilizing interlocking fingers. These fingers create a sensing region that winds through the electrode fingers, as indicated by the dotted line below in Figure 8.

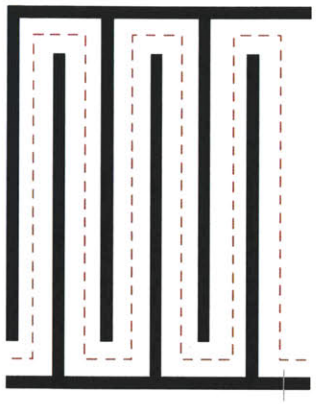

Figure 8: Sensing Region Between Interdigitated Electrode Fingers [32]

With a constant gap width throughout the electrode, the sensing region can be modelled based on the characteristic length (the total length of the dotted line) and gap along the sensing area. The proportion of length over gap (L/g) is inversely proportional to the impedance. Reduction in the length or increasing the gap between fingers will increase the impedance. This relationship makes the repeatability of finger width and spacing critical to the repeatability of the impedance drop measurements. Even small variations to finger dimensions or defects have affected the proportion of length and gap, and therefore the resulting impedance drop measurements.

The rest of this chapter describes how the testing methods were performed, and how the electrodes' robustness tests to shipping, handling, and production defects were performed and analyzed. The tests were designed to investigate the impact of defects and handling on the sensing area, and therefore the repeatability of the electrodes.

4.2 Testing

At Daktari, testing with solutions of known conductivity was necessary to calibrate the design of the electrode pattern. Before this work, there were two setups to test the behavior of the electrodes. These tests were designed to emulate the behavior of the electrode in the final system without needing a completely finished cartridge to test.

4.2.1Testing Methods:

The first testing method was the assay development platform (ADP). This was a clear injection molded PMMA chip with a die cut adhesive. The adhesive is used to form the assay chamber and attach the electrode to the chip. Solutions of known conductivity were flowed through the assay chamber at a rate of 20 μl/min with the assistance of a syringe pump. Figure 9 shows the ADP Testing Setup. The details of the ADP chip are easier to see in Figure 10.

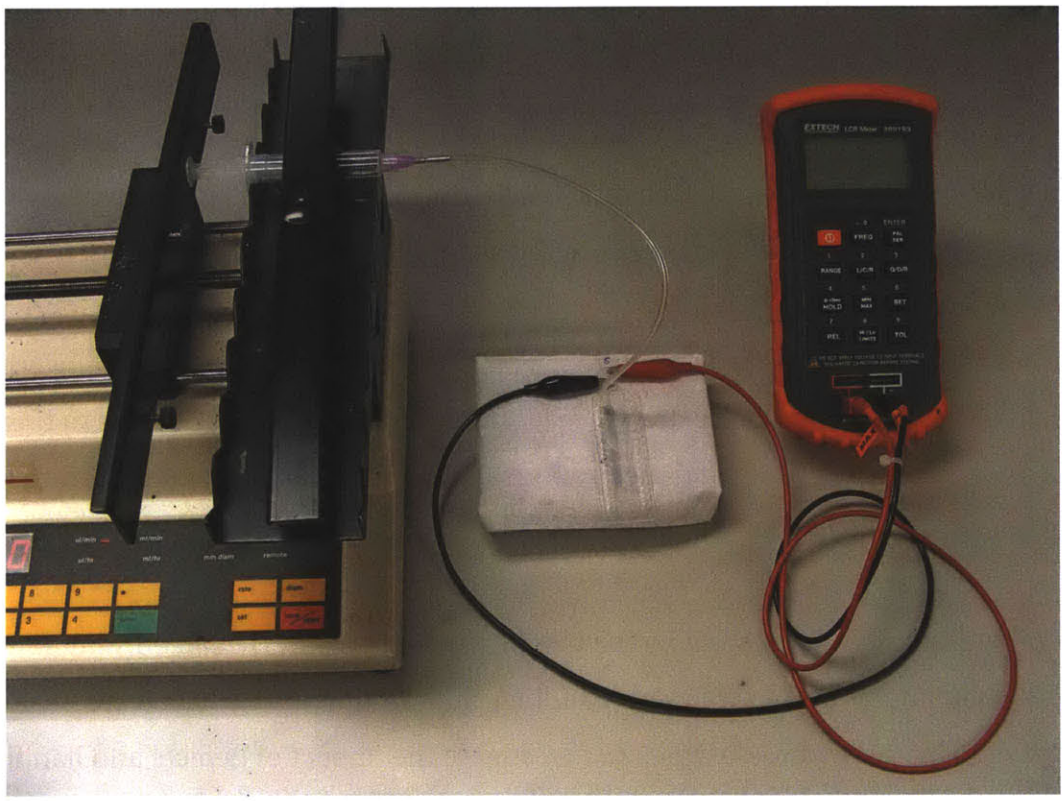

Figure 9: ADP Chip Testing Setup

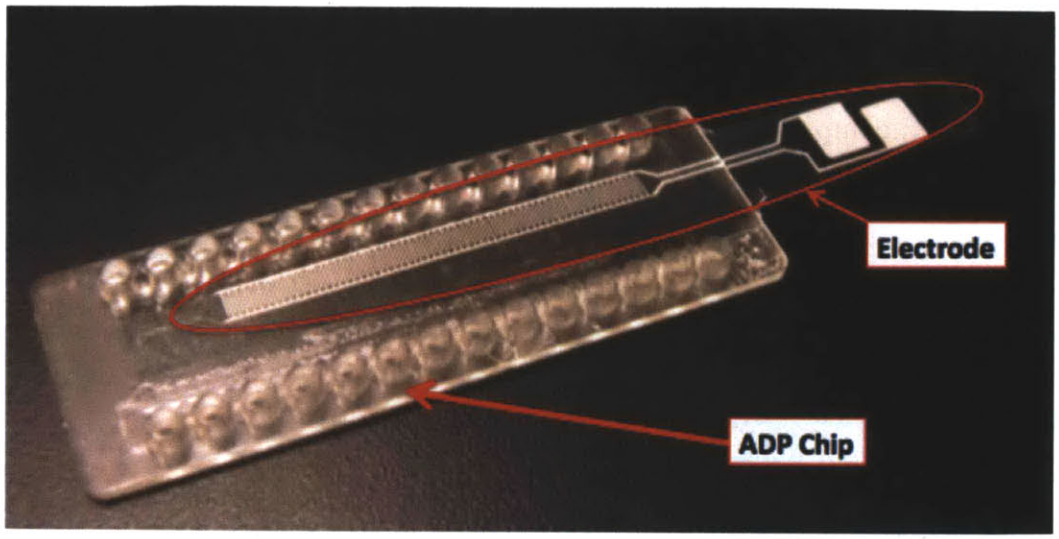

Figure 10: Up-Close of ADP Chip and Electrode

The impedance is read from a meter attached to the connector pads on the electrode. Once the impedance stabilizes, the flow is stopped for two minutes, and the impedance is recorded. Using different solutions a linear relationship can be found between inverse of the impedance and the solution conductivity. Throughout the remainder of this document the slope of the linear fit is used to characterize the relationship and variability between electrodes.

This testing method can be rapidly assembled. The shape of the assay chamber is similar to the production system. But this method does have disadvantages. The adhesive used to create the assay chamber and attach the electrode to the ADP chip adds noise to the output reading in the form of ions. Ions leach out of the adhesive into the chamber and change the conductivity of the input solution. The ions from the adhesive requires that initial cleaning step before the test can be performed. The cleaning step must be done overnight by running deionized water through the chip at a rate of 5 μl/min to flush out the ions from the adhesive.

A second testing method uses a subassembly of the cartridge. The electrode and a lid foil are laser welded to the backbone. Tape is used to seal unnecessary openings and vents. Solutions of known conductivity are plumbed through the assay chamber at a rate of 20

μl/min. Similar to the ADP chip, the impedance is read from a meter attached to the connector pads on the electrode. Once the impedance seems stable, the flow is stopped for two minutes and the impedance is recorded. Using different solutions, the slope of a linear relationship can be found between the inverse of the impedance and the solution conductivity.

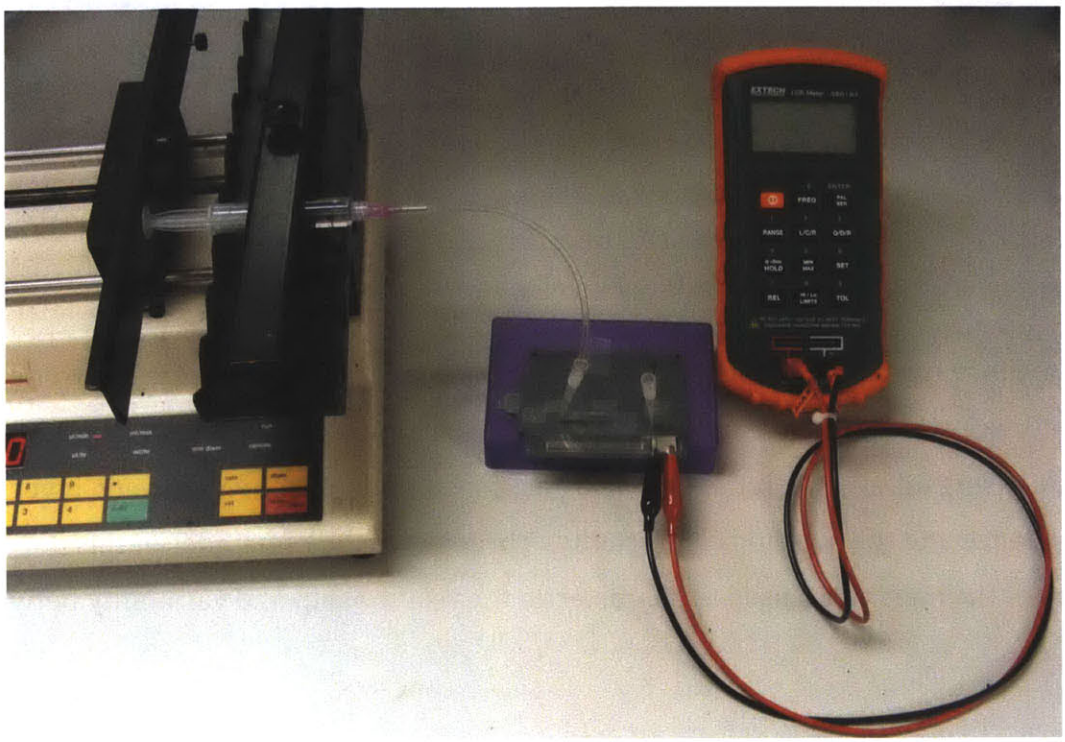

Figure 11: Card Subassembly Test Setup

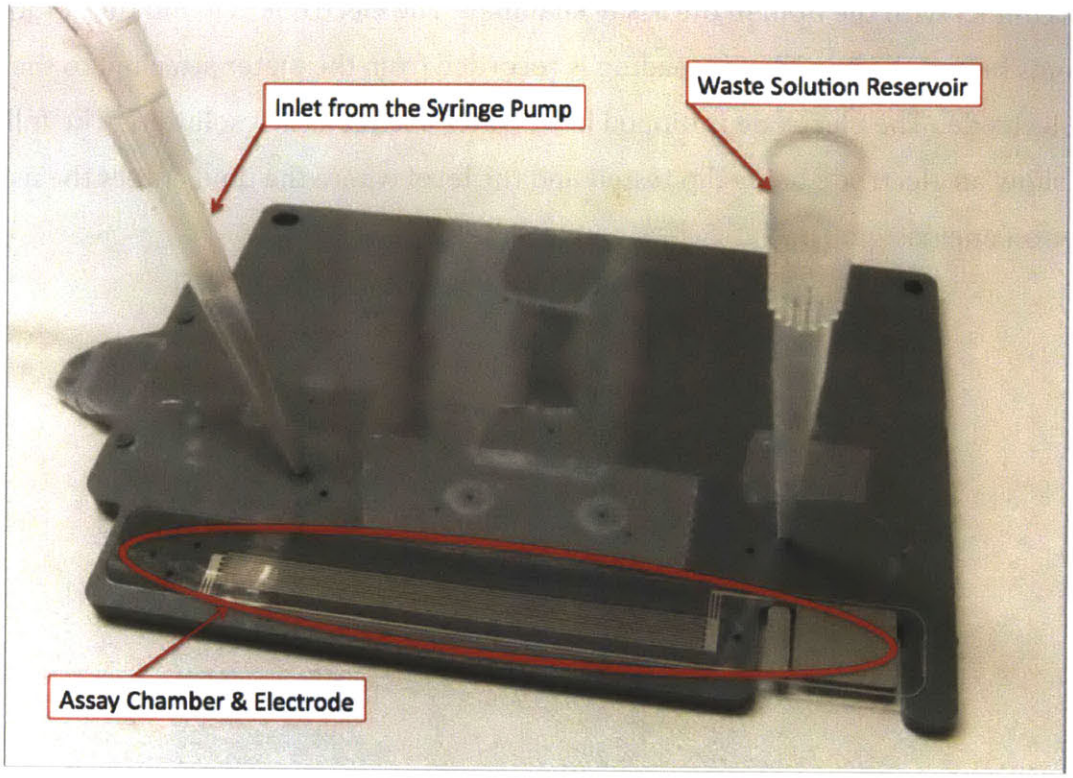

Figure 12: Up-Close View of the Card Subassembly

This method is a subassembly of the cartridge with only the necessary components to test the electrode foil. The electrode and lid foil are welded to the backbone so there is no adhesive to add ions to the system. There is no need to run a cleaning solution overnight. Therefore, data is faster to obtain than the ADP chip method after the cards are produced. Unfortunately, there is a long lead-time because the backbone must be molded, and the electrode and lid are attached to the backbone with a laser weld in Germany.

4.2.2 Dip Test Method:

The first goal of the thesis project was to develop a new method that can speed up testing. The previous test methods required excessive time either to be cleaned or to be produced. The new method needed to be quick and easily done at Daktari's facility.

The "Dip Test" is performed by taking an electrode rinsed with deionized (DI) water and submerging it in solutions of known conductivity to the level where the electrode would

come in contact with the fluid in the assay chamber. The electrode is left in the solution for 10 seconds before the impedance reading is recorded from the meter attached to the pads on the electrode. The electrode is dipped in DI water between each solution. The following figures show an electrode being dip tested and the level where the fluid enters the assay chamber on one design.

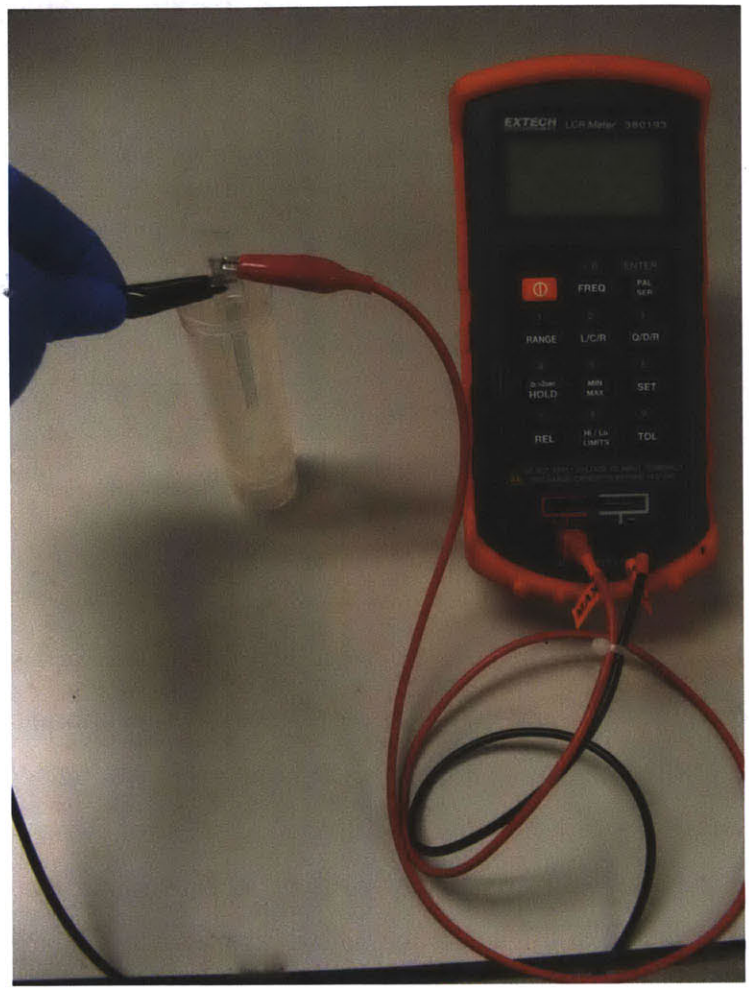

Figure 13: Dip Test Method Setup

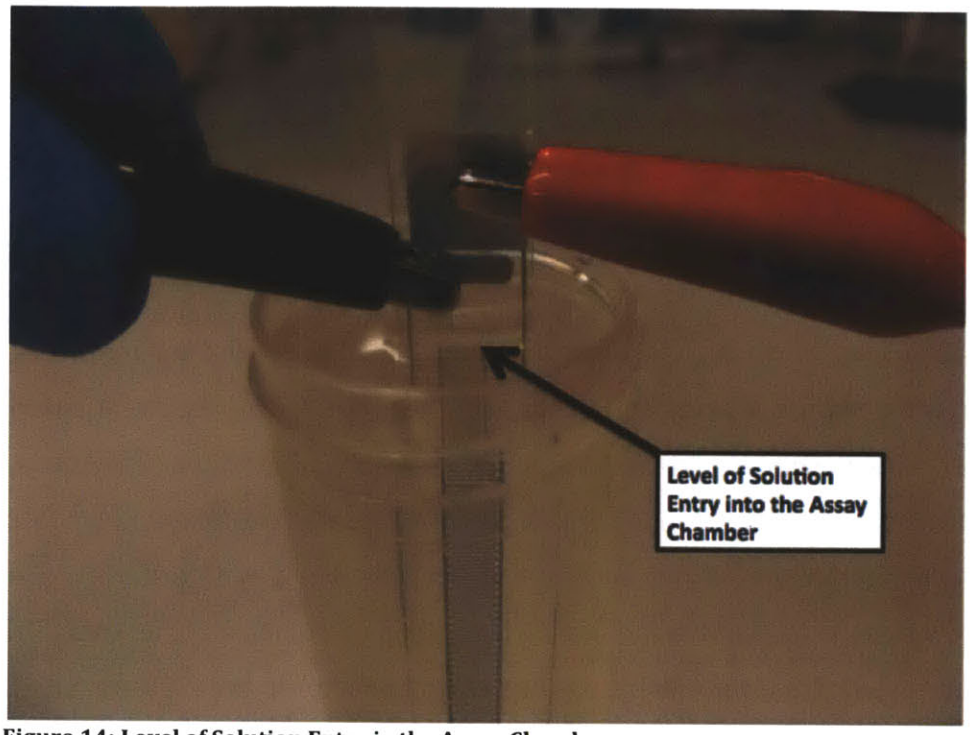

Figure 14: Level of Solution Entry in the Assay Chamber

The dip test was used in this thesis because an electrode can be tested without being attached to another component. Each data point can be measured within seconds so this allows for larger sample sizes. However, the electrode is being dipped into a larger volume of water, results from the dip test do not equal the results seen from other testing methods. Therefore, the dip test is used as a preliminary testing procedure to get a qualitative understanding about the electrodes because the dip test isn't quantitatively the same as other testing methods.

Because the dip test was a new testing method, the repeatability of the test had to be determined. The repeatability tests were performed by dip testing a single electrode ten times in the same solution. This test was repeated for five solutions of increasing conductivities. This procedure estimates how accurate a single impedance measurement was for a given solution conductivity.

Most of the work for this thesis was concerned with the accuracy of the slope of the inverse of the measured impedance divided by conductivity, a single electrode was dip tested with

all five solutions going from low conductivity to high conductivity. The electrode was washed with fresh DI water after each set of five solutions. The series of five solutions was repeated ten times. The variability of the slopes was then determined. This experiment was used to determine the lowest possible standard deviation possible in the slopes for the dip test.

Because initial dip tests were giving different slopes than the other two methods, a relationship between the dip test and the card subassembly test was investigated. Two different electrode designs were dip tested and welded to a backbone. The intercept of inverse of the measured impedance divided by conductivity relationship was forced through zero for both tests. From the results a relationship was determined. Close-ups of the two patterns used are shown in Figure 15 and Figure 16 below. The two different patterns were used to insure that the pattern wasn't a factor in the relationship. These patterns have fingers that run perpendicular to each other and have different slopes. The first pattern is the one used for the rest of the tests in this thesis. This design was used because it was the most optimized design with the new production method, and this pattern is similar to the pattern of the gold electrodes.

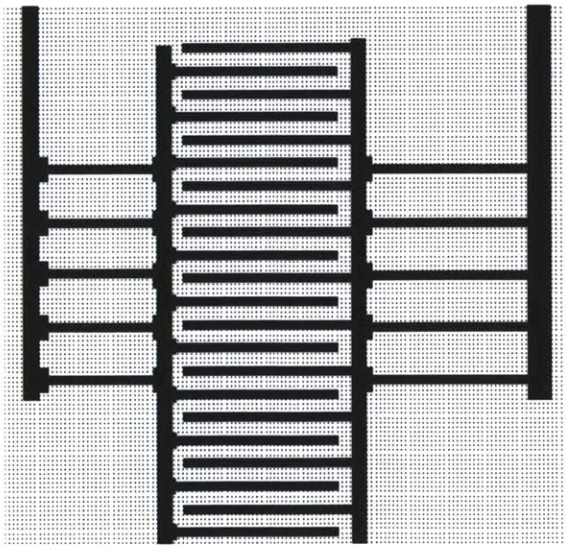

Figure 15: Close-Up of Short Finger Design [22]

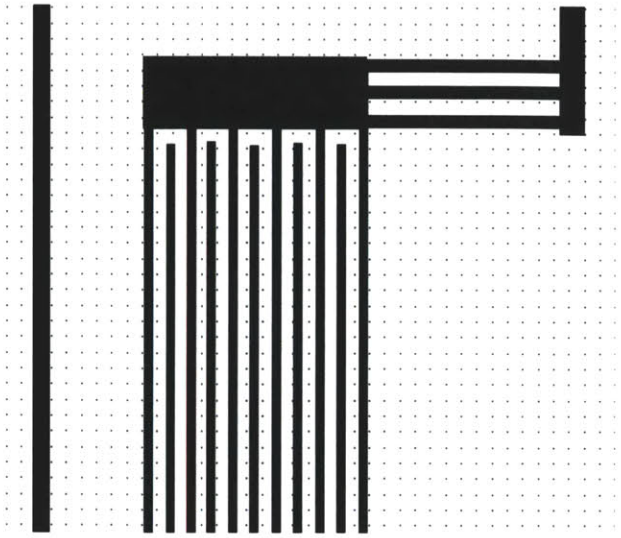

Figure 16: Close-Up of Long Finger Design [22]

4.2.3 Test Accuracy

Error was measured as percent cell equivalent error. This metric is how two electrodes with different slopes and variations were compared. The way the instrument uses the slopes is as follows:

An unknown input number of cells proportionally relates to an input conductivity.

1. The actual electrode doing the measurement reads the input conductivity.
2. The inverse of the impedance is measured with the actual electrode.
3. The inverse of the measured impedance is used with the average value line.
4. The output conductivity is then measured.

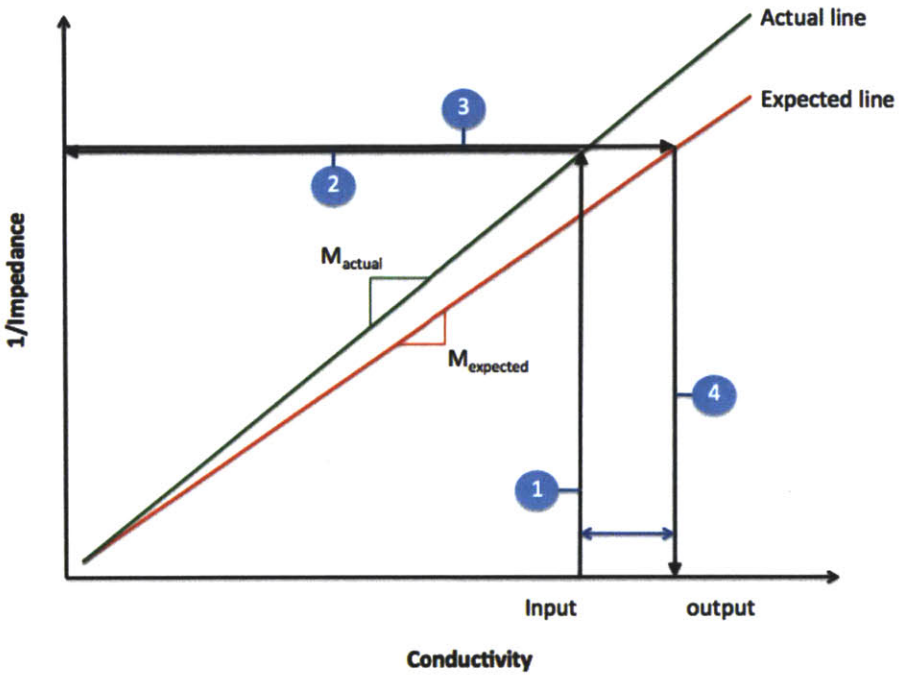

Figure 17: Procedure for Determining % Cell Equivalent Error

Using the equation of a line y = mx+b and both the actual and expected lines go through zero so b=0.

$$\frac{1}{impedance} = Input_{conductivity} \times M_{measured} = Output_{conductivity} \times M_{expected} \quad (1)$$

$$Output_{conductivity} = Input_{conductivity} \times \frac{M_{measured}}{M_{expected}} \quad (2)$$

$$\%CellError \equiv 1 - \frac{M_{measured}}{M_{expected}} \quad (3)$$

The measured slope can be steeper or shallower than the expected slope. Ideally if all the electrodes have the same slope the percent cell error would be zero. However, the actual slopes will give positive or negative percent cell error.

4.3 Electrode Robustness

The new process made electrodes that were more robust than the gold version. The following testing was performed on the new electrodes to quantify the robustness to handling, shipping, and defects.

4.3.1 Shipping and handling

The new electrodes are qualitatively more robust than the gold electrodes, but quantitatively investigating the robustness to physical abuse is necessary to develop tolerances and guidelines for shipping and handling.

There were five types of physical abuse that were identified as areas of concern. The first two were shipping methods. Shipping was simulated for loose electrodes in two containers by shaking the containers. The two containers were a cardboard box and a plastic Petri dish. The slope and percent cell error for these two methods was compared to the control electrodes that did not go through a shipping simulation.

The next three types of handling were bending, twisting, and rubbing the electrodes. Bending the electrode means bending it about an axis parallel to the width shown in Figure 19. Twisting the electrode is putting the electrode in torsion by ±180 degrees about the centerline down the length of the electrode. The twisting motion is shown in Figure 19. The final type of abuse was rubbing two electrodes together with force of 1.47 N shown in Figure 20.

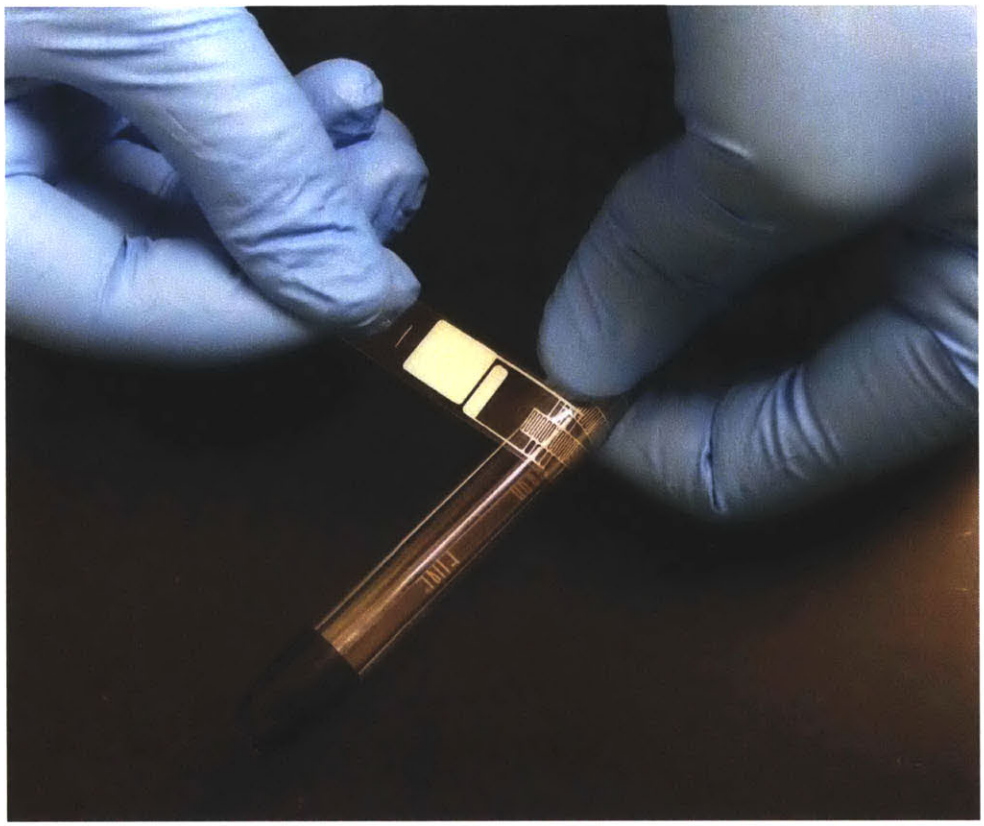

Figure 18: Bending the Electrode

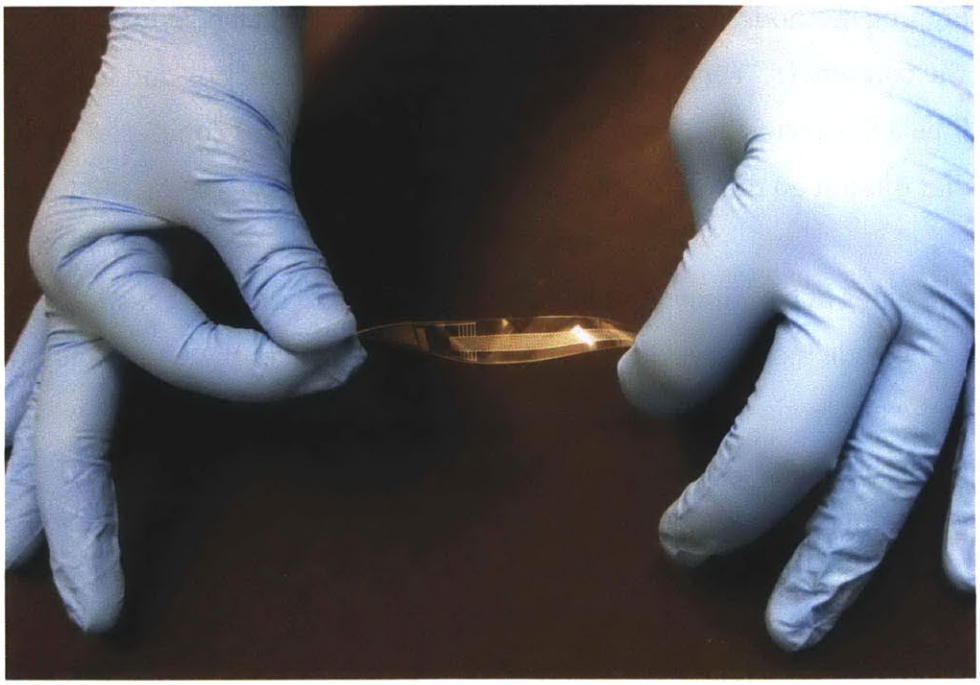

Figure 19: Twisting the Electrode

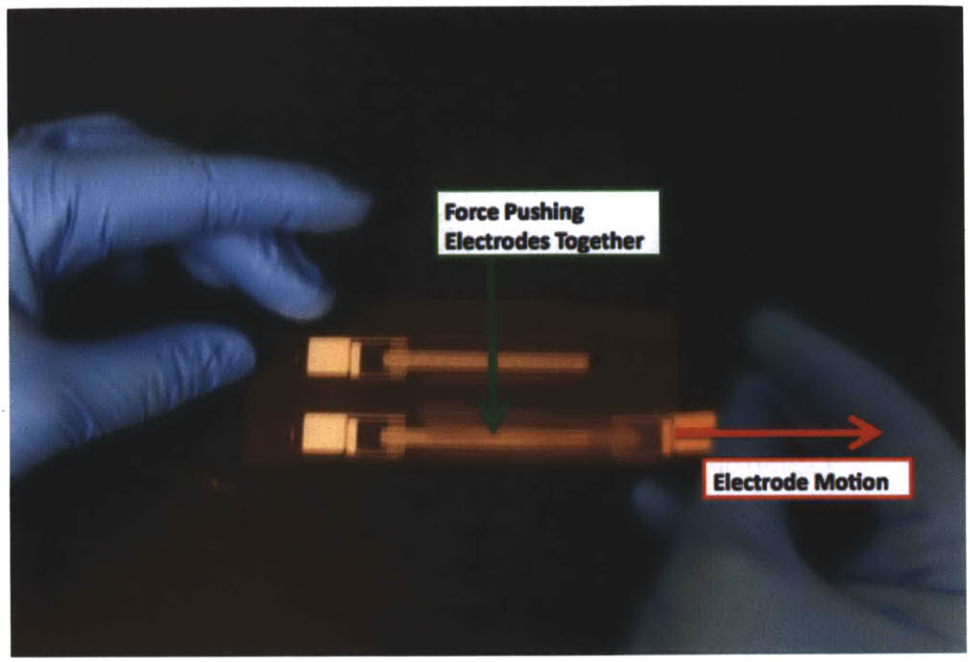

Figure 20: Rubbing Electrodes Together

The production order is shown in Table 1 along with the testing order. The testing order was randomized to eliminate any potential variance because of trends during production or testing. The codes of letters and numbers indicate the markings that were used to identify the electrodes. A total of 25 electrodes were tested.

Table 1: Production and Testing Order for Shipping and Handling Tests

Production Order		Testing Order	
Control	C1	Bent	B4
Card Board	CB1	Card board	CB2
Plastic Dish	PD1	Control	C4
Bend	B1	Bent	B3
Twist	T1	Control	C1
Control	C2	Card board	CB4
Card Board	CB2	Control	C2
Plastic Dish	PD2	Twist	T1
Bend	B2	Card board	CB5
Twist	T2	Plastic Dish	PD4
Control	C3	Twist	T4
Card Board	CB3	Card board	CB1
Plastic Dish	PD3	Twist	T5
Bend	B3	Bent	B2
Twist	T3	Plastic Dish	PD5
Control	C4	Control	C5
Card Board Box	CB4	Plastic Dish	PD2
Plastic Dish	PD4	Card board	CB3
Bend	B4	Twist	T3
Twist	T4	Control	C3
Control	C5	Twist	T2
Card Board Box	CB5	Plastic Dish	PD3
Plastic Dish	PD5	Plastic Dish	PD1
Bend	B5	Bent	B1
Twist	T5	Bent	B5

4.3.2 Robustness to Defects

The variability caused by defects was examined, and a Design of Experiments (DoE) methodology was used to explore four factors. The first factor was the type of defect: production errors or scratch errors. The production error is when a connection is never produced. Scratch errors come from handling. The second factor was the location of the defect on the finger. The defects were either half way down the length of the finger or between the finger and the side rail. The third factor was the defect location in the electrode. The defects were in the half close or in the half away from the connection pads. The fourth factor was the number of defects. The number of defects was 5, 10, 15, or 20. Table 2 shows the testing order for the DoE. The experiment tested 32 electrodes with five solutions of different conductivities to determine the conductivity slope.

Table 2: Defect DoE

Type	Finger Location	Quantity	Electrode Location
Production defect	Base	5	Towards connectors
Scratch	Middle	5	Away from connectors
Production defect	Base	20	Away from connectors
Scratch	Middle	20	Away from connectors
Production defect	Middle	20	Towards connectors
Production defect	Middle	5	Towards connectors
Scratch	Middle	15	Towards connectors
Scratch	Base	20	Towards connectors
Scratch	Middle	5	Away from connectors
Production defect	Middle	20	Towards connectors
Production defect	Base	10	Towards connectors
Scratch	Base	5	Away from connectors
Scratch	Base	10	Away from connectors
Production defect	Base	20	Away from connectors
Scratch	Base	5	Away from connectors
Production defect	Middle	15	Away from connectors
Production defect	Middle	10	Away from connectors
Production defect	Base	15	Away from connectors
Scratch	Middle	15	Towards connectors
Production defect	Middle	5	Towards connectors
Scratch	Middle	10	Towards connectors
Production defect	Base	15	Away from connectors
Scratch	Middle	20	Away from connectors
Production defect	Middle	15	Away from connectors
Scratch	Base	10	Away from connectors
Scratch	Base	15	Towards connectors
Scratch	Middle	10	Towards connectors
Production defect	Base	10	Towards connectors
Production defect	Middle	10	Away from connectors
Scratch	Base	15	Towards connectors
Production defect	Base	5	Towards connectors
Scratch	Base	20	Towards connectors

After the initial testing, more investigation was done on the defect location within the electrode and the type of defect. The first six fingers and last six fingers were produced without a connection to the side rail. Removing the fingers from each end of the electrode was done to characterize an extreme failure case. The patterns tested are shown below in Figure 21 and Figure 22.

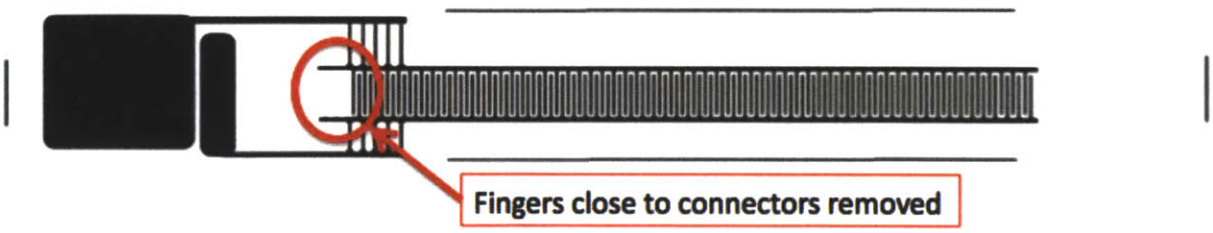

Figure 21: Missing Fingers Close to the Connectors

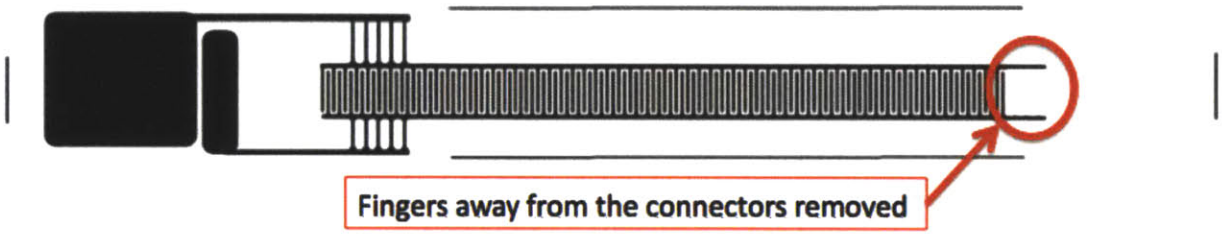

Figure 22: Missing Fingers away from the Connectors

There was concern that all scratches may not actually sever fingers. To verify that scratches do have the same effect as a production error, good electrodes had the first six or last six fingers severed from the side rail. The same fingers were scratched in the same place as the production error and verified as a complete break under the microscope. The slopes were measured with the dip testing method and compared. This test was done in collaboration with the previous test. The testing order for is shown in Table 3.

Table 3: Testing Order of Defect Type & Defect Location on the Electrode

Testing Order
SA 5
PA 3
PA 4
C 1
PT 1
ST 1
SA 2
PT 2
C 2
ST 4
PA 1
SA 4
SA 1
PA 5
ST 2
ST 5
PA 2
SA 3
PT 3
PT 4
C 4
C 3
ST 3
PT 5
C 5

The first letter stands for the type of defects. Scratch defects are noted with an S, and print defects are noted with a P. The second letter indicates if the defect was away (A) or towards (T) the connector pads. Control electrodes are identified with C. The numbers represent the production order.

The final concern that was investigated was the impact of broken finger remnants on variation in the electrode slopes. Electrodes were produced with the same fingers broken or completely missing. The two types of electrodes were dip tested to find the slopes. The testing order is shown in Table 4 and also the codes and electrode description.

Table 4: Missing vs. Broken Fingers Testing Order

Code	Electrode Description
2d	Missing Finger Electrode 4
1c	Broken Finger Electrode 3
2c	Missing Finger Electrode 3
1e	Broken Finger Electrode 5
2e	Missing Finger Electrode 5
2b	Missing Finger Electrode 2
2a	Missing Finger Electrode 1
1a	Broken Finger Electrode 1
1f	Broken Finger Electrode 6
1b	Broken Finger Electrode 2
1d	Broken Finger Electrode 4
2f	Missing Finger Electrode 6

The slopes were compared, using a t-test, to see if there was a significant difference between them. Below in Figure 23 and Figure 24, the patterns of broken and missing finger electrodes that were tested are shown. The same 15 fingers are defective in both patterns. The only difference between the electrodes is the finger remnant after the break.

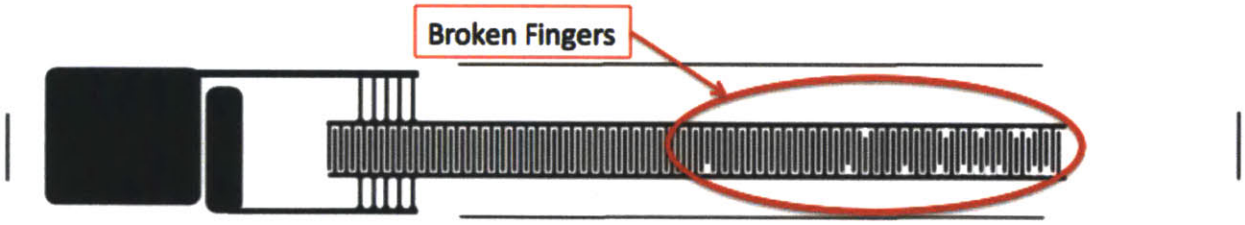

Figure 23: Electrode with Broken Fingers

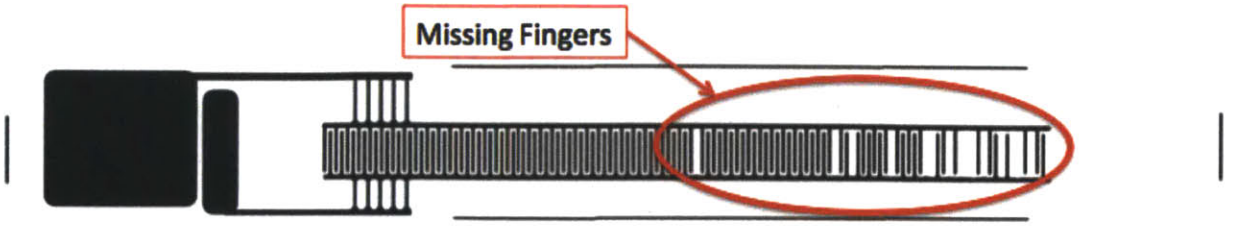

Figure 24: Electrode with Missing Fingers

Chapter 5: Results & Discussion

5.1 Dip Test Repeatability

The electrode tested for the repeatability of the slope measurement had an average slope of 1.44×10^{-5} cm/characteristic length with a standard deviation of 8.5×10^{-8} cm/characteristic length. For this specific electrode pattern, the repeatability of the dip tests was found to be ±1.8% cell equivalent error. The percent cell equivalent error depends on the conductivity slope for the particular electrode. The data from this test is shown in Figure 25.

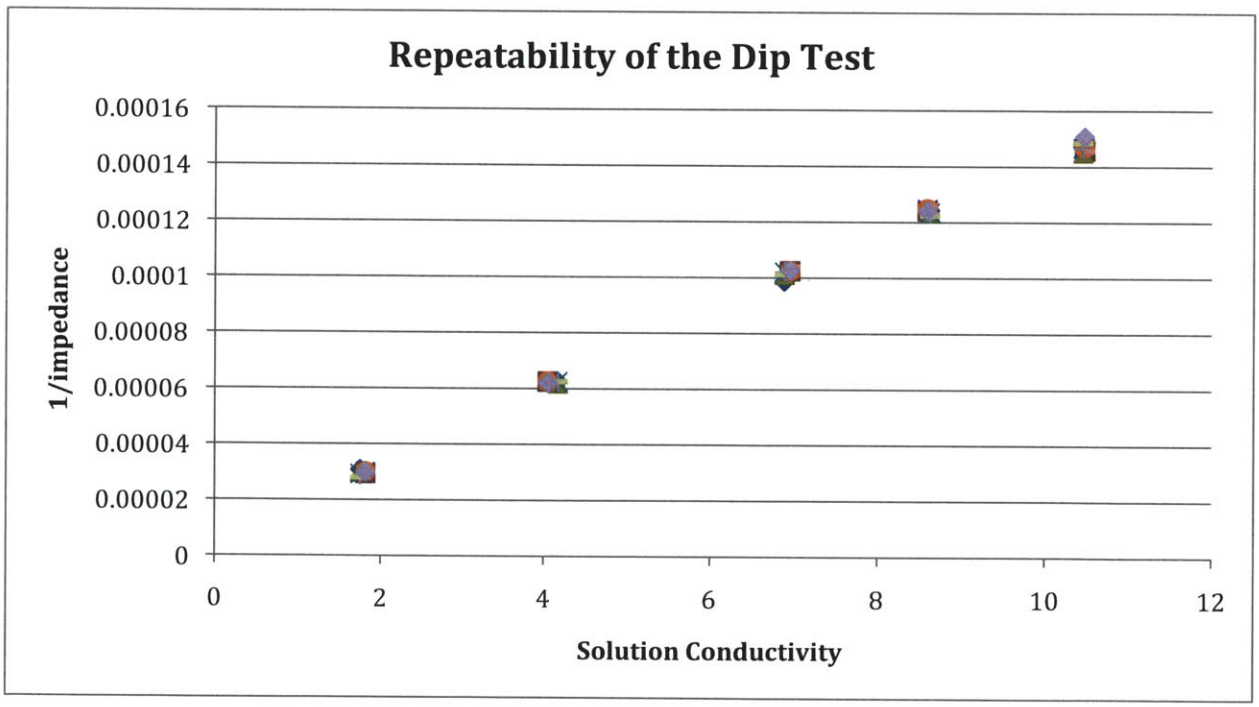

Figure 25: Dip Test Repeatability Data

The slope variability is much lower than the variability in each measurement point because the slope is determined with many measurements and some errors will cancel out. The meter attached to the electrode measures the resistance in kΩ. The variability in the resistance measurement is different at different input conductivities. Table 5 shows a summary of the results. Single data points were observed as far as ±10% away from the average measurement at low conductivities and about ±3% for higher conductivities.

Table 5: Single Point Repeatability

Average input conductivity (μS/cm)	1.716	4.105	6.95	8.625	10.35
Average resistance (kΩ) measured	34.41	16.072	9.915	8.083	6.776
Standard Deviation of Resistances (kΩ)	0.899	0.081	0.104	0.045	0.053

5.2 Relationship Between Tests

A clear relationship between the dip test and the subassembly test was found. The two different patterns, shown in Figure 15 and Figure 16, were tested using both the welded card subassembly method and the dip method. The pattern with long fingers gave the purple data with the highest slope when it was dip tested and the red squares when tested with the subassembly. The pattern with short fingers gave the green triangle data when dip tested and the blue diamonds when tested with the card subassembly. The dip test gave a slope that was twice as steep as the card subassembly test for both patterns. The dip test method uses a much larger volume of fluid than the welded card method. The extra fluid has ions near the electrode that reduce the impedance by increasing the size of the electric field. All of the intercepts are fixed at zero. Figure 26 shows the data graphed. The result means that the dip test slope results can be used to predict the slope in the welded card subassembly.

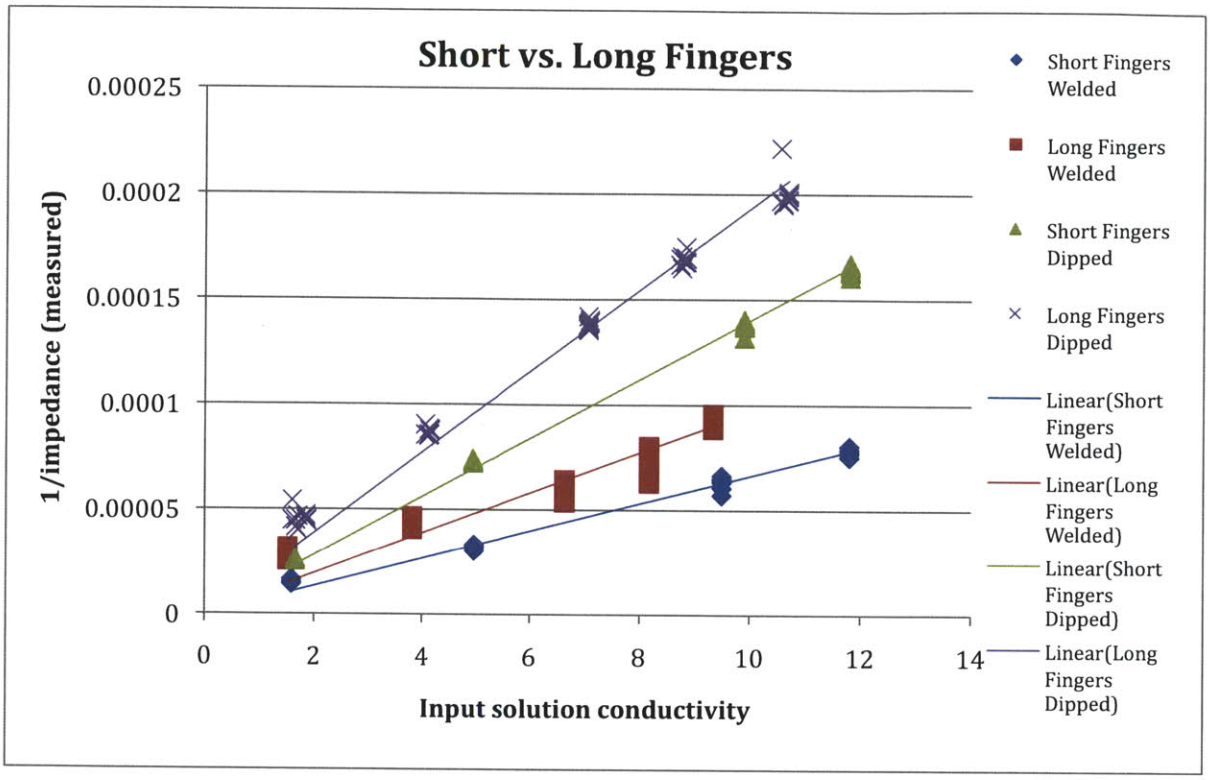

Figure 26: Dip Test & Welded Subassembly Data

5.3 Electrode Robustness

5.3.1 Shipping & Handling

The tests for electrode robustness to physical abuse resulted in only one treatment that is significantly different from the control electrodes with a 95% confidence interval. Figure 27 shows where the slope of each electrode lies within its treatment. The bending raised the average slope and clustered the data points closer together. This result was unexpected because defects in the electrode usually cause the slope to drop, or for the results to have a wider spread in the slopes.

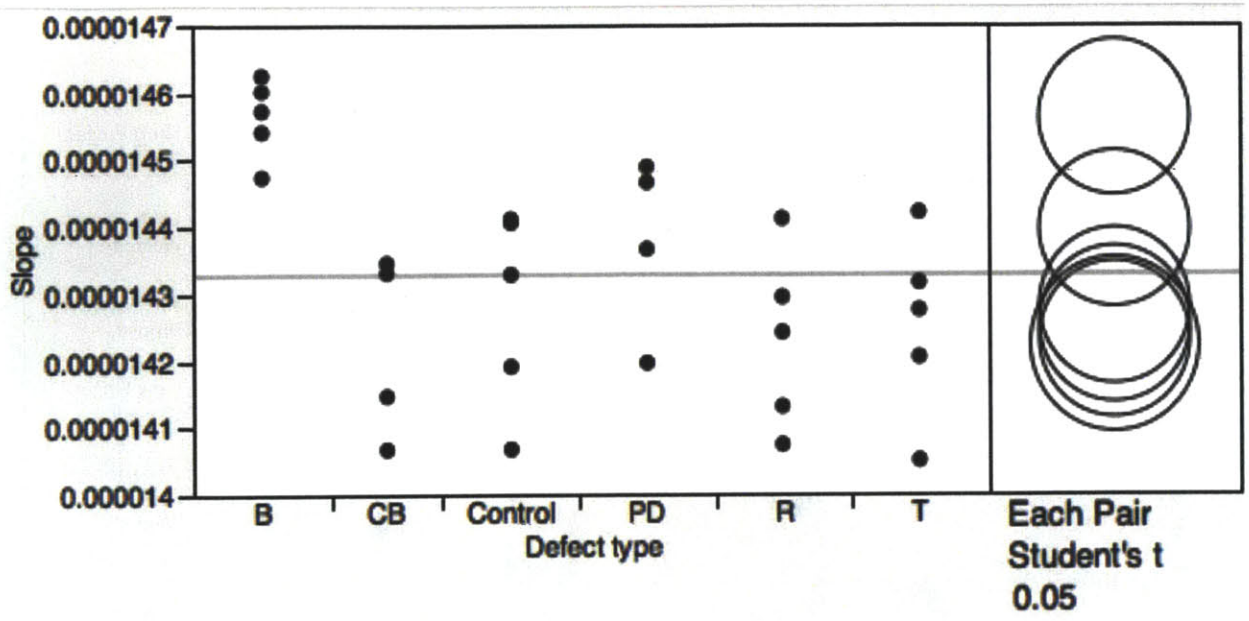

Figure 27: Shipping & Handling Results

One theory to explain the electrodes' response is that the bending introduces microcracks on the electrodes' surface. Microcracks may aid in the wetting process and remove leftover conductive material from the production method. Unfortunately, Daktari may not be able to take advantage of this behavior because the electrodes need to lie flat when the antibody solution is applied. Bending the electrodes should be avoided because it changes the slope and may prohibit the application of antibody solution with the current antibody application method.

Another theory is that bending the electrodes causes the material to stretch. Stretching can also help remove the leftover conductive material without making the electrodes bowed. This should be tested in the future to see of the company can take advantage of the behavior seen in the bending case.

The only treatment that resulted in a nonperforming electrode was the cardboard shipping simulation. One electrode had a small scratch along the side rail that wasn't noticed during the initial visual quality inspection under the microscope. These new electrodes may be

robust, but some care should be taken to immobilize the electrodes during shipping to reduce the possibility for damage.

5.3.2 Robustness to Defects

The results of the next set of testing tell if the electrode performance is susceptible to various defects. Information from this set of testing can be used to establish appropriate tolerances for quality control. The DoE used for this test was not a full factorial. Sixteen unique populations were tested with one replicate for a total of 32 electrodes tested.

The number of defective fingers significantly correlates to the drop in the electrodes' slope. There was no significant difference between 10 and 15 breaks and between 15 and 20 breaks according to the t-test at a 95% confidence interval. It was expected that the number of breaks would be proportional to the drop in the slope of the electrodes, and this trend was seen in the data from the DoE. The trend appeared to be approximately linear. Figure 28 shows the results from the DoE for the number of fingers. There is variation in the data because the DoE was testing four factors, but a trend can be seen, and t-tests confirm that there is a significant difference.

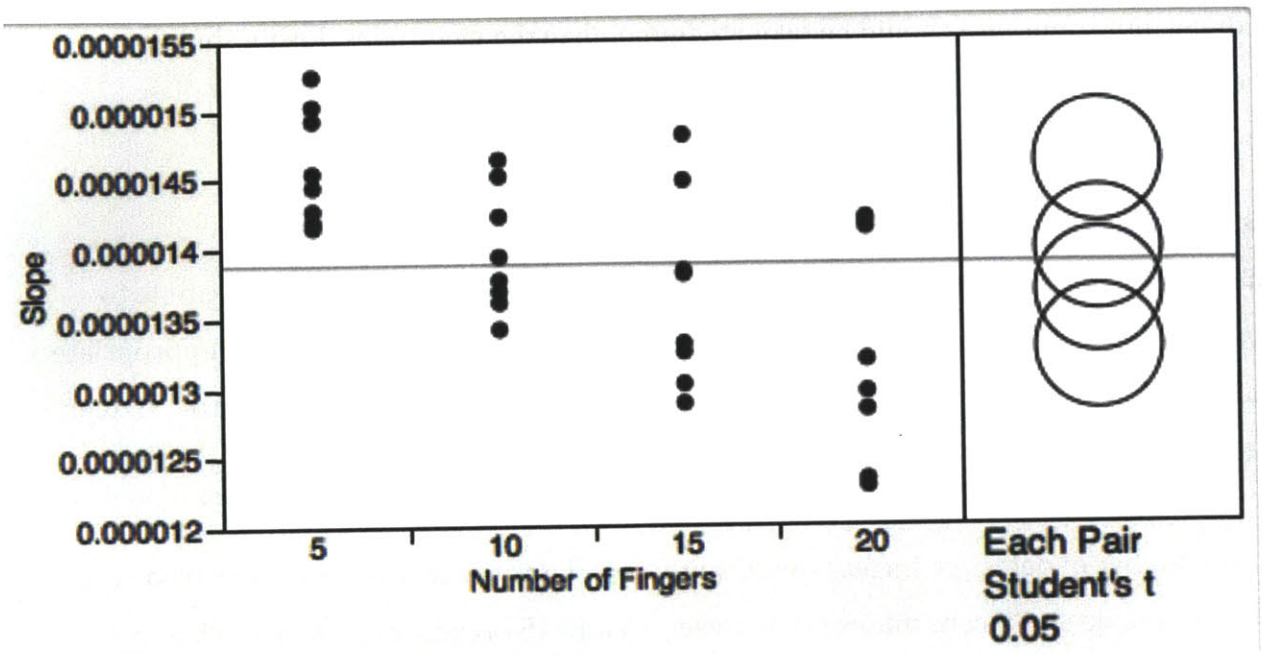

Figure 28: Number of Finger Break Results

Table 6 relates the percent drop in the average slopes between the populations and compares it to the percent drop in the number of fingers. When looking at the comparison the percent drop in slope seems closer to the percent drop in number of fingers when there are 5 fingers with defects. The defective fingers were chosen at random. Since the fingers are chosen at random some fingers were broken close together, and when the fingers are broken close together the impact of each break is reduced. This reduction of the impact explains why the percent drop in slope does not increase as fast as the percent drop in the number of fingers.

Table 6: Drop in Slope and Drop in Number of Fingers

	% Drop in Slope	% Drop in the Number of Fingers
From 5 defects to 10 defects	3.91%	3.97%
From 10 defects to 15 defects	2.26%	3.97%
From 15 defects to 20 defects	3.38%	3.97%
From 5 defects to 15 defects	6.08%	7.94%
From 10 defects to 20 defects	5.56%	7.94%
From 5 defects to 20 defects	9.25%	11.90%

Statistically significant results were not obtained for the break location on the finger. However, looking at the results there seems to be a slightly greater reduction in slope because of the breaks at the side rail. This response is expected because a break at the side rail and the finger removes more sensing area from the electrode than a break in the middle of the finger. This effect is minor because the difference in the sensing area is not changed enough to give significant results. Figure 29 shows the results from the DoE.

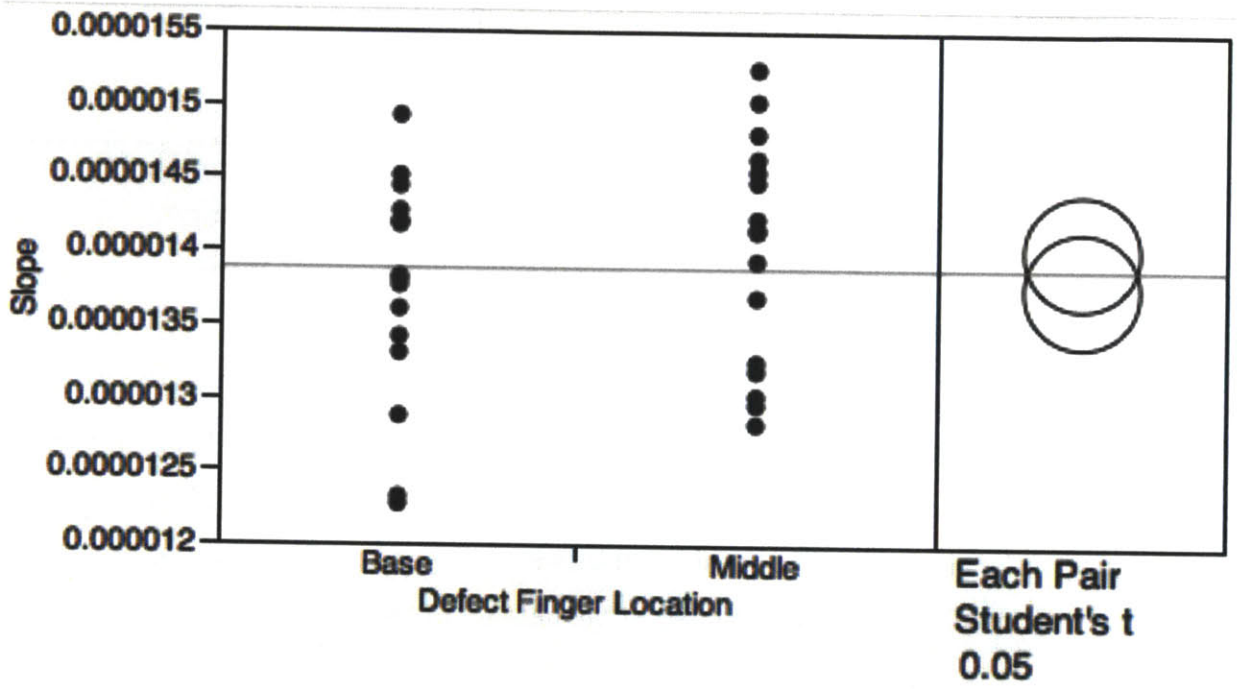

Figure 29: Defect Location on the Finger Results

Production defects were found to drop the slope significantly more than the scratch defects according to the results from the DoE shown in Figure 30. This result is understandable because scratches may not always sever the finger. The gold electrodes also experienced scratches that didn't cause an electrical break. The scratches, under further examination, looked like perforations instead of a clean cut. The impact of complete scratches that broke electrical continuity was explored more in the next set of testing.

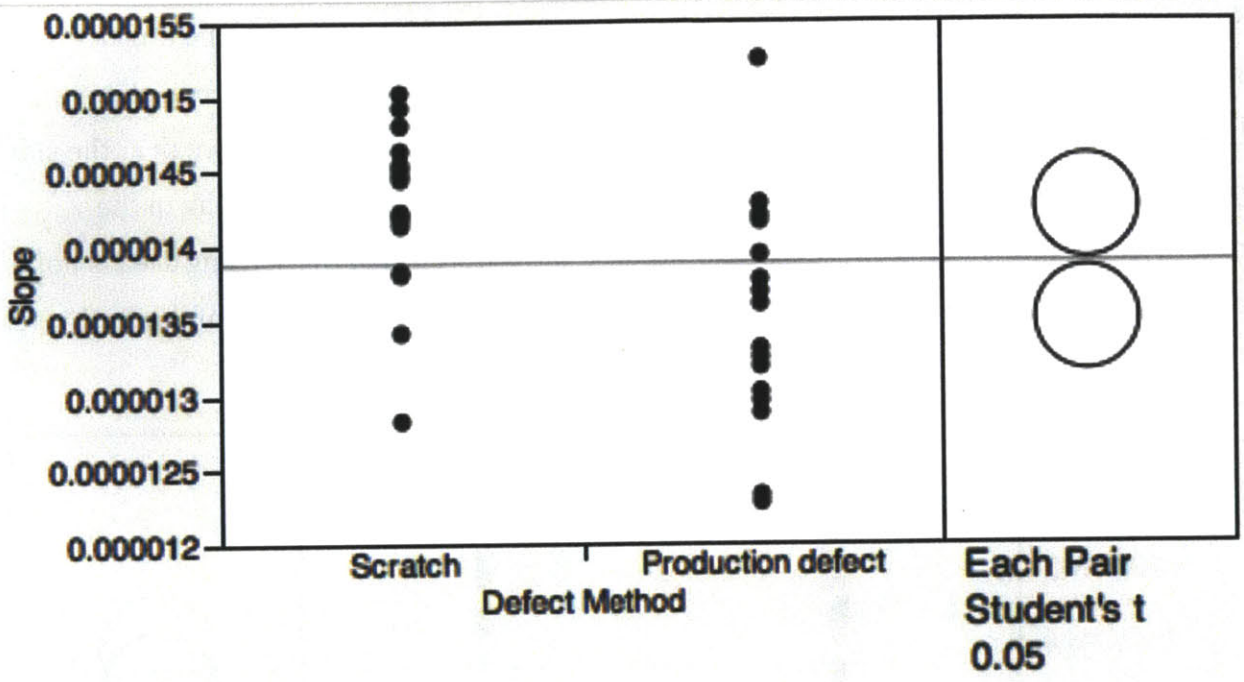

Figure 30: Defect Method Results

The difference between the break locations on the electrodes was not significant. However the results suggest that defects closer to the connection pads cause less damage to the performance of the electrode. The results can be seen in Figure 31. The cause of this behavior is unknown, but was investigated in the next set of testing.

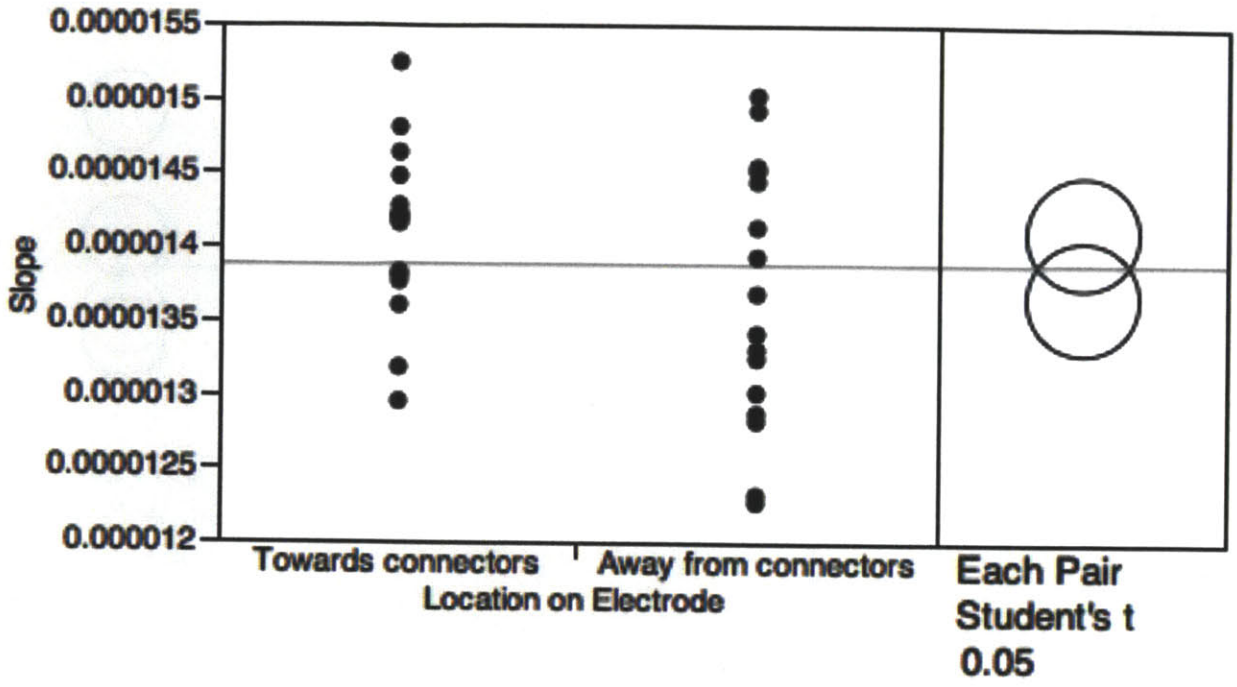

Figure 31: Defect Location on the Electrode Results

5.3.3 Further Testing

After the DoE was completed, the "Further Testing" experiments were performed to focus on questions that arose from the previous results. The new test suggested that production defects were not as sever as scratch defects, which is the opposite conclusion from the earlier testing. The difference in the conclusions is shown comparing Figure 30 and Figure 32. In Figure 32 the scratch defects seemed to drop the slope more than the production defects. The likely cause of this is that while insuring that the scratches severed a finger, extra minor damage could have been done to the electrodes. This result suggests that any defect that severs electrical continuity changes the behavior of the electrodes in the same way.

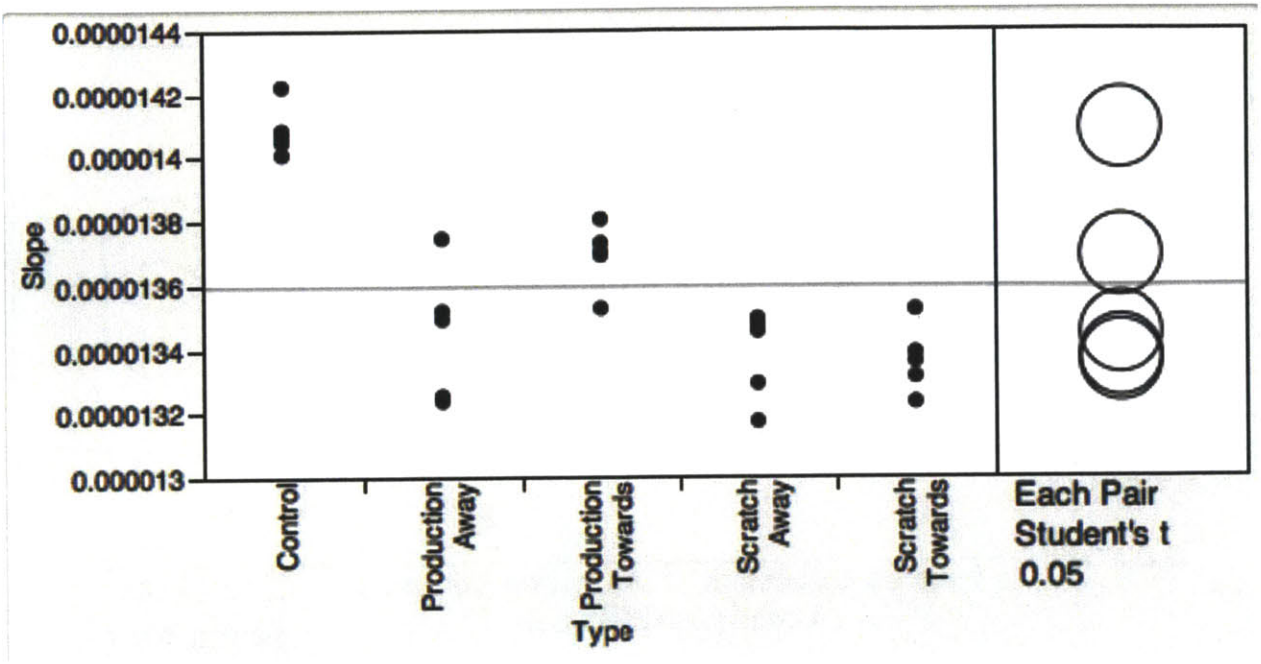

Figure 32: Further Testing Results

In the DoE, defects towards the connectors and defects away from the connectors were on random fingers in the respective zones. In further testing, the first six fingers or last six fingers have defects to maximize the difference between the two conditions. In Figure 32 it can be seen on both the production and scratch defects that the defects further away from the connectors have a slight effect on the slope. This result from further testing agrees with the previous DoE. The cause of the difference between defects close or away from the connectors isn't verified. The probable cause of this difference is that there is less current further down the electrode so the defects disrupt the electrode more than close to the connection pads where the current is stronger. However, currently this impact isn't significant enough to warrant a design change. If this effect significantly degrades the ability of the electrode to perform well in the cartridge, the design can change to be symmetric so one end isn't closer to the connectors than the other end.

In the final test, the broken fingers and missing fingers were compared; there is no significant difference between the two populations. In Figure 33 it can be seen that the data points and trend lines are clustered with respect to each other. This agrees with other results that there is no difference between a small break or scratch and a missing finger. A

t-test was performed on the data and there is no significant difference between the populations. If electrical continuity is broken, it can be modeled as if there was no finger produced.

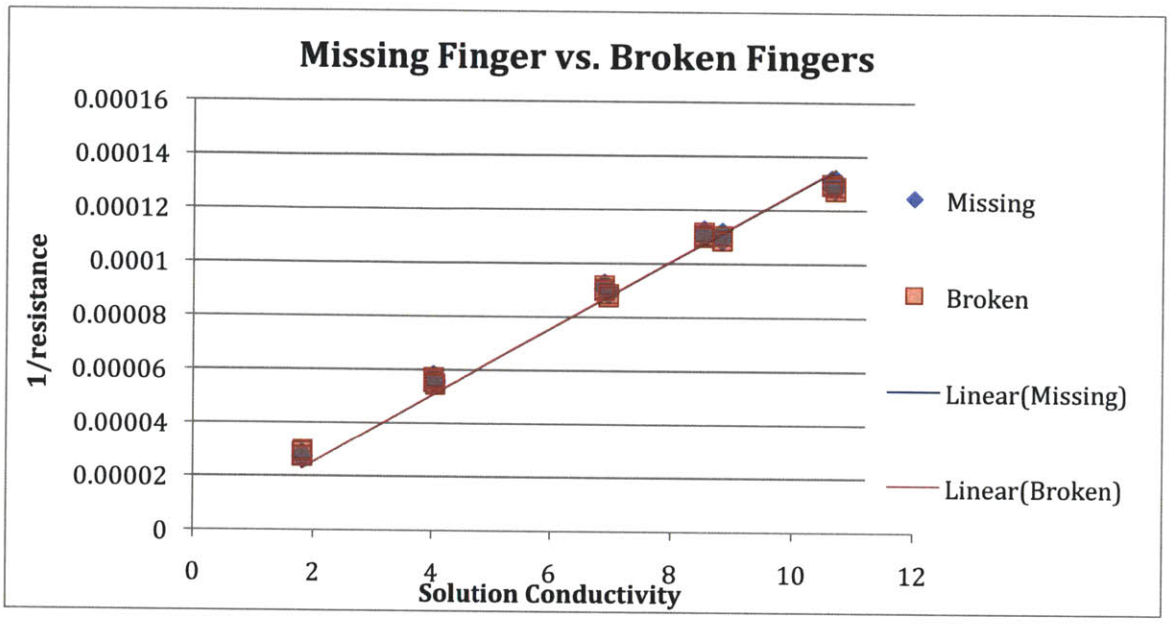

Figure 33: Missing Finger vs. Broken Finger Data

Chapter 6: Conclusion & Recommendations

In conclusion, the dip test was found to be a useful and accurate rapid testing method. The accuracy of the test is high enough to obtain useful results that allow the user to draw conclusions from the data about how the electrode will perform in the cartridge. Because the new production method is so flexible this testing method is fast enough to help guide Daktari Diagnostics to produce the best electrode possible.

However, the dip test does not replace the card subassembly test for understanding how the actual system will function. In the full system there are concerns with the assay chamber filling and antibody function. The dip test only gives insight with electrical performance and relative scale of the slope.

This thesis project found that the most significant defect was the number of defective fingers in the electrode. The more defects present the more the electrode performance deviates from the expected slope.

Bending the electrodes was also found to affect the slope and may cause problems with the process steps to apply the antibody and weld the electrode to the backbone. Bending should be avoided during production and in any handling steps after, unless there is a way to process the electrodes before the addition of antibody to insure that the electrodes will remain flat for the final processing steps.

The new process is producing robust electrodes compared to the old process, but care should still be taken in order to insure that defects are avoided and the test gives the most accurate results possible.

The testing was completed to understand the robustness of the electrodes to defects, shipping, and handling should be repeated specifically for the final electrode pattern that

will be used in the cartridge. It is unknown at this point if the pattern can affect the durability of the electrode.

Chapter 7: Future Work

7.1 Process Parameter Optimization

Work was done by Donoghue and Holmes [33] in early 2011 to optimize the process parameters. This work should be expanded on to optimize the parameters for the electrode design that Daktari Diagnostics moves forward with for the clinical trials or very low volume production.

7.2 Robustness of New Designs

The new manufacturing process is flexible and new designs were explored by Donoghue [22]. The robustness of these designs to defects, shipping, and handling should be characterized before moving to development of a quality control device in order to identify significant defects.

7.3 Functionalization

The ability to adhere the antibody to the electrode and achieve acceptable cell capture is vital for the performance of the device. The functionalization process needs to be tested and confirmed that it is attaching the antibody to the electrode foil. This adhesion must be done without degrading the antibody's ability to capture the CD4 cells.

7.4 Flow Characteristics

Once the functionalization process is performing at an acceptable level, the flow characteristics of the blood and reagents must be observed through the assay chamber. Proper filling of the chamber is necessary for cell capture and the elimination of air bubbles. Design changes may need to be made to the shape of the assay chamber or the location of the antibody on the electrode foil in order to achieve proper filling.

7.5 Ageing Study

The final step before the new electrodes can replace the gold electrodes, an ageing study must be done to insure that the component remains viable and accurate throughout the

duration of its shelf life. This should be done by accelerated ageing in an oven designed for ageing tests with samples monitored on a regular basis throughout the duration of the test.

7.6 In-Line Quality Control

Once production is ramped up for these electrodes an in-line quality control testing method should be in place to monitor the electrodes. This device will depend on the electrode production method, electrode pattern, and type of test it will perform.

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