I. Project Title
A Paper-Based ELISA Device for The Rapid Detection of Ischemic Stroke

II. Project Completion Date
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III. Student(s), Department(s), and Major(s)
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V. Cooperating Industry, Agency, Non-Profit, or University Organization(s)
N/A

VI. Executive Summary
Diagnosing an ischemic stroke accurately and quickly is essential for appropriate treatment and results in more effective management of the stroke, leading to better patient outcomes. Specifically, the determination of ischemic versus hemorrhagic stroke leads to critically different treatment paths, which would be potentially fatal if administered incorrectly. Currently, ischemic strokes are diagnosed through a series of tests, including a physical examination, CT scans, and a panel of blood tests to exclude hemorrhagic stroke or other similarly presenting conditions, in order to administer tPA (the main treatment) within the three hour window.

The discovery of biomarkers, molecules which are upregulated during the onset of a specific condition or disease, can be used to diagnose patients when integrated with an appropriate platform. The objective of this thesis was to further develop a paper-based microfluidic device for the rapid detection of ischemic stroke using biomarkers that can be detected in the blood. This device uses HRP-based enzyme linked immunosorbent assay (ELISA) technology on a cellulose paper surface to yield a sensitive (11.8 pM) assay targeting S100B, a protein released by glial cells in the brain during stroke. Wax printing allows for creation of precise hydrophilic pathways in which sample fluid can travel within the 3D microfluidic device to react with a variety of proteins and reagents.

This technology has the potential to be implemented globally as a point-of-care device for use in developing countries without consistent or reliable access to advanced diagnostic technology. The device is cheap to produce, portable, and results can be determined quickly and qualitatively analyzed using cell phone images, making it an accessible technology for patients around the globe.
VII. Major Accomplishments

(1) Designed a device to route fluid on a 3D cellulose- and nitrocellulose-based platform using wax printing techniques to form hydrophobic barriers, thereby creating hydrophilic channels of paper in which the sample fluid would travel. The layers of the device can be seen in Figure 1. The various designs were characterized and tested originally using red dye in order to gain a fundamental understanding of the device functionality. A housing was designed for the device and laser cut out of acrylic at the Cal Poly Mustang ‘60 Machine Shop in order to further improve layer connectivity and ease-of-use for the end user, as seen in Figure 2.

(2) Demonstrated ELISA-like capability of the device using on-board reagents incorporated onto the paper surface in order to react with and bind to the target analyte and produce a visible colorimetric response for a positive test, as seen in Figure 3. Used proteins and reagents purchased using Baker Koob Endowment funds as outlined below in Section VIII to fully display device functionality to detect the target protein in a sample.

(3) Used smart phone images of the device and computer software to analyze and quantify the concentration of target analyte in the sample. ImageJ was used to quantify the color intensity in order to develop a sigmoidal curve for signal-to-noise versus protein concentration. This was then used to calculate a limit of detection for the device which allows for the determination of clinical significance of the device. An LOD of 147.9 pg/ml (11.83 pM) was achieved.

Figure 1. Individual paper layers of the device printed with wax and cut out for assembly. (A) Cellulose paper layers, from top left: sample introduction pad, wash layer, fluid routing layer, absorbent pad. (B) Nitrocellulose layer for detection and control regions. (C) Glass fiber pads for storage of the conjugate antibody.
Figure 2. Completed device made from acrylic and wax-printed paper layers. (A) Device housing with screws inserted used to hold top and bottom layers together. (B) Fully assembled device aligned to accept sample in sample ports.

Figure 3. Final results of a device running a series of protein concentrations which were analyzed and plotted versus color intensity. (A) The device run with high protein concentrations and one control, displaying the blue color for a positive result which can be correlated to concentration. (B) Dose response graph for a range of concentrations. Error bars = ± 1 SD. Logarithmic (base 10) scale used on X-axis.

VIII. Expenditure of Funds
The entirety of the funding was used to purchase reagents and proteins that were used to realize the full ELISA-like functionality of the device to test for a target protein and display a colorimetric response. Without these funds, the device would only have been validated to the point of fluid connectivity, and the funding allowed the project to advance to a clinically significant result, meeting and exceeded the original goals of the project.

IX. Impact on Student Learning
This thesis allowed me to gain a hands-on and personal realization of how impactful POC diagnostic development is and how important the field is for healthcare in general. I was able to integrate what I had learned from my engineering experience on device development, design, and
manufacturing in conjunction with my understanding of biology and immunologic reactions in order to produce a functioning device. It was extremely impactful for me to have the ability to use the proteins and reagents purchased with the Baker Koob Endowment funds to create a device which could be used to detect and diagnose ischemic strokes faster than ever before. I gained valuable experience in experimental design and implementation, data analysis, and, most importantly, learning how to overcome obstacles and problem solve to ultimately yield a significant final result. I have taken this experience and all that I learned to my new position at a POC diagnostic device company where I will further continue my work in assay development.