Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

CAL POLY

Proposal Cover Page

Title of Project:
Portable and Low-Cost Paper-Based Microfluidic Assay Device for Stroke Detection.

Proposal Author: Andrea Larsen **Cal Poly Email:** anlarsen@calpoly.edu

Student ID: 009926926 **Dept.:** CENG - BMED

Signature (Optional): Andrea Larsen 11/5/2018
Signature provides permission to check financial aid eligibility.

Previous Baker/Koob Endowment funding? (circle one): Yes (No)

Is this request to support a Senior Project or thesis? (circle one): (Yes) No

Team Member(s)	Signature	Cal Poly Email	Department
Andrea Larsen	Andrea Larsen	anlarsen@calpoly.edu	CENG - BMED

Anticipated Start Date: September 20, 2018 **Anticipated End Date:** June 1, 2019

Total Funds Requested: $2,413

Faculty Advisor: Dr. David Clague **Department:** CENG - BMED

Faculty Advisor email: dclague@calpoyl.edu **Telephone:** (805) 756-5145

Signature of Faculty Advisor: David D. Clague **Date:** 11/5/18

PROPOSAL NARRATIVE

I. Project Title

Portable and Low-Cost Paper-Based Microfluidic Assay Device for Stroke Detection.

II. Abstract

In emergency rooms today, a CT scan is the standard for assessing the presence and extent of neurological trauma. Unfortunately, without contrast solution, CT scans miss ischemic strokes, which account for ~80% of strokes [1]. In addition, when considering developing countries, there is limited, if any, access to expensive CT imaging. Therefore, a stroke will not be diagnosed efficiently, which would increase the risk of long-term complications and even death. However, during and after a stroke, there are detectable macromolecular signals, biomarkers, in the blood stream that can be used to diagnose the disease [2].

The project described in this proposal is a master's thesis involving the development of 3D paper-based microfluidic devices to enable ELISA assays. This project will further develop a low-cost, portable lateral flow assay device for early detection of stroke via blood biomarkers. One of the main objectives of this project is to create a device that maximizes ease of use by non-experts. A key issue in the development of a lateral flow assay is addition of onboard reagents to eliminate reagent handling. Thus, a major outcome of the project is to incorporate these conjugate reagents in the prototype. To reach that outcome, $2,413 of funding is requested to purchase antibodies and antigens that can detect stroke biomarkers, purchase device supplies, and to sponsor a trade conference.

III. Objectives

The goal of this master thesis project is to develop a paper-based microfluidic assay which can flow fluid through predefined channels across a conjugate pad loaded with an onboard reagent and reach the test strip of the assay in an efficient manner.
The main objectives are as follows:

1. Design and develop a prototype paper-based microfluidic assay device.
2. Further develop strategies to enable onboard conjugate reagents.
3. Experimentally validate the design of the device via smart phone image analysis.

Lateral flow assays are used for a variety of applications, including pregnancy tests, urine glucose tests, or tests for diseases like malaria, dengue, and typhoid fever [3]. The patient sample is spotted onto the assay sample pad and then runs along a path, leading it across the conjugate pad where the sample solubilizes the detector reagent with labeled antibodies. These antibodies bind to the biomarkers in the sample and later bind to capture antibodies on the test line to display the results of the assay [4]. Assays are currently developed in several forms to target specific biomarkers. In general, these assays are set up similar to that seen in Figure 1, below.

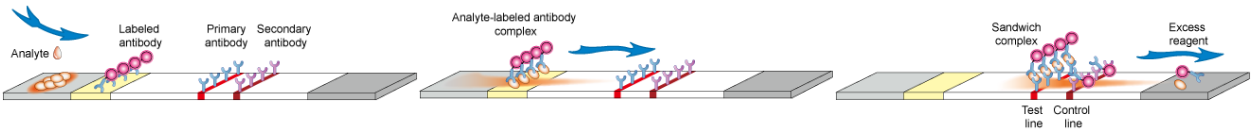

Figure 1. Lateral Flow Assay Functionality (Creative Diagnostics, (n.d.))

The reagent that is loaded on to the yellow strip shown above, the conjugate pad, includes a labeled antibody that will bind to the target analyte in the sample. The issues surrounding this specific design feature are the preliminary loading and eventual solubilizing of the conjugate reagent on the pad. The further development of the device to include this reagent will allow for minimal sample preparation and reduce user error in a clinical or research setting. When applied to multiplex assays, which assess for several different biomarkers, test efficiency and accuracy improve, creating smoother processes for the user and better outcomes for the patients.

This project will focus on using several CAD models to optimize the pathways for fluid flow, wax printing to enable device prototyping, and functional testing to validate the device design. The expected conclusion is a thesis, in tandem with a fully functional lateral flow assay device that can efficiently flow fluid across a conjugate pad loaded with a reagent. While not directly interdisciplinary due to it being a single-student thesis, this project incorporates facets of many fields. Primarily, the project is based on principles from biomedical engineering, including aptamer/target molecule binding, capillary flow as related to biological systems, and general assay functionality. It does also incorporate fluid dynamics, optics, and CAD design from mechanical engineering and an understanding of principles from materials engineering, biology, human physiology, and chemistry.

IV. Methodology

In order to complete the objectives listed above, a series of tasks, mapped in the timeline in Section V, and the corresponding deliverables have been defined. The project will begin with a literature review to determine which technologies exist in this space and what types of developments are being made with these assays. I will be looking specifically at capillary flow publications, material properties and surface characterization literature, and conjugate reagent loading methods. With this knowledge I will run iterations of the CAD design of the wax pathways for the fluid flow and eventually down-select my proposals. Then, I will move on to the manufacturing of assay prototypes using the wax printer, loading the conjugate reagents, and overall assembly of the full assay using housing from previous projects. I will also design validation experiments to have the proper methods set up to determine the efficacy of the fluid flow across the assay and conclusion on if the reagent was loaded properly to be solubilized.

Once my prototypes and the experimental designs are completed, I will proceed with performing the experiments and run statistical analysis on the data collected. The efficacy of the design will be validated experimentally using antibodies and antigens with known interactions as well as dye flow to develop the device. The verification will determine if the reagent on the conjugate pad is solubilized correctly and carried to the test line. At this point, I may need to redesign based on experimental results and run through the process an additional time to account for what I learned from the experiments. When the redesign and verification pass is complete, I will complete my thesis report and defense.

V. Timeline

Below in Figure 2, the proposed weeklytimeline from September 20th, 2018 until the

project completion on June 1st, 2019 with divisions for each major deliverable and subsections for large tasks that need to be completed.

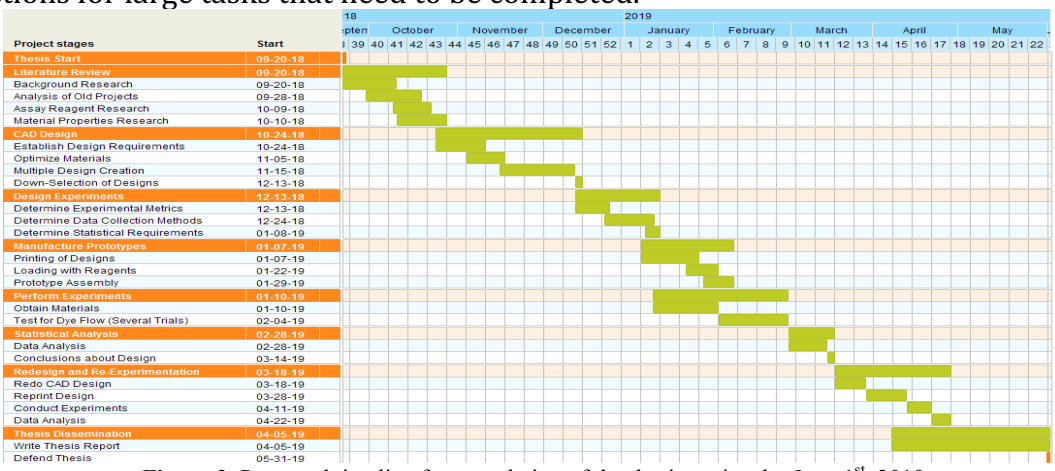

Figure 2. Proposed timeline for completion of the thesis project by June 1st, 2019

VI. Final Products and Dissemination

The final form of this project will be a completed master's thesis. I plan to disseminate my results in the form of a written report and a presentation at my thesis defense. The final prototype is expected be used in future master's thesis to continue the design process and improve other components of the assay. I will also investigate into presenting at professional conferences for biotechnology, such as the Lab on a Chip conference.

VII. Budget Justification

The total cost breakdown for the project is shown in the table below. Primarily, I am requesting funding for materials to functionally test my device. Using antibodies and antigens to detect the proteins that are seen in clinical practice is vital to validate my design and elevate my thesis. The GFAP protein has been tied to stroke diagnosis by several studies and is an excellent macromolecule to use to test the final device prototype [6] [7]. The membranes and chromatography paper will be used for the physical build of the device and deliver a platform on which the sample will be released and the conjugate reagent, including the antibodies, will be loaded. The streptavadin will be used as a binding agent to aid the Anti-GFAP antibody to bind to the nitrocellulose membrane.

Item No.	Vendor	Item	Cost	Justification
1	Millipore Sigma	Nitrocellulose Membrane	$ 300.00	Membrane to allow for antibody conjugate reagent immobilization and assay creation
2	Millipore Sigma	Cellulose Acetate Membrane	$ 204.00	Membrane to allow for antibody conjugate reagent immobilization and assay creation
3	abcam	Recombinant Human GFAP protein (ab114149)	$ 435.00	Human GFAP for testing - sample protein
4	abcam	Anti-GFAP antibody (Alexa Fluor® 488) (ab194324)	$ 455.00	Antibody for detecting binding of human GFAP
5	Millipore Sigma	Anti-GFAP Antibody, Biotin Conjugate | MAB3402B	$ 412.00	Antibody for capturing human GFAP to the chip
6	Thermo Fisher	Strepavidin Protein (PI21122)	$ 147.00	Protein to bind capture antibody to the chip
7	Millipore Sigma	Chromatography Paper	$ 60.00	Chromatography paper for the assay to be built on to allow the sample to travel
8		Conference Fees	$ 400.00	Registration fees for a biotech conference - Lab on a Chip
		TOTAL COSTS	**$2,413.00**	

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PROPOSAL BUDGET

Student Applicant(s): Andrea Larsen		
Faculty Advisor: Dr. David Clague		
Project Title: Portable and Low Cost Paper-Based Microfluidic Assay for Stroke Detection		**Requested Endowment Funding**
Travel *subtotal*		**$ -**
Travel: In-state		
Travel: Out-of-state		$ -
Travel: International		$ -
Operating Expenses *subtotal*		**$ 2,413.00**
Non-computer Supplies & Materials		$ 2,013.00
Computer Supplies & Materials		$ -
Software/Software Licenses		$ -
Printing/Duplication		$ -
Postage/Shipping		$ -
Registration		$ 400.00
Membership Dues & Subscriptions		$ -
Multimedia Services		$ -
Advertising		$ -
Journal Publication Costs		$ -
Contractual Services *subtotal*		**$ -**
TOTAL		**$ 2,413.00**

APPENDIX A

REFERENCES

[1] Musuka, T. D., Wilton, S. B., Traboulsi, M., & Hill, M. D. (2015). Diagnosis and management of acute ischemic stroke: speed is critical. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 187(12), 887-93.

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[4] Christopher P., Robinson N., Shaw M.K. (2005) Antibody-Label Conjugates in Lateral-Flow Assays. In: Wong R.C., Tse H.Y. (eds) Drugs of Abuse. Forensic Science and Medicine. Humana Press

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[6] Ren, C., Kobeissy, F., Alawieh, A., Li, N., Li, N., Zibara, K., Zoltewicz, S., Guingab-Cagmat, J., Larner, S. F., Ding, Y., Hayes, R. L., Ji, X., … Mondello, S. (2016). Assessment of Serum UCH-L1 and GFAP in Acute Stroke Patients. Scientific reports, 6, 24588. doi:10.1038/srep24588

[7] Foerch C, Curdt I, Yan B, Dvorak F, Hermans M, et al. (2006) Serum glial fibrillary acidic protein as a biomarker for intracerebral haemorrhage in patients with acute stroke. J Neurol Neurosurg Psychiatry. 77(2): 181–4.