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

FINAL REPORT

Final reports will be published on the Cal Poly Digital Commons website(http://digitalcommons.calpoly.edu).

I. Project Title

Development of a Lateral Flow Assay (LFA) for the Rapid and Cost-Effective Diagnosis of Cerebrospinal Fluid (CSF) Fistulae

II. Project Completion Date

January 15th, 2020

III. Student(s), Department(s), and Major(s)

(1) Megan Mitchell, Department of Biological Sciences, Biological Sciences Major

(2) Brandon Strong, Department of Biological Sciences, MS in Biological Sciences

(3) Emily Christensen, Department of Biological Sciences, Biological Sciences Major

IV. Faculty Advisor and Department

Andres Martinez, M.D., Ph.D., Chemistry Department

V. Cooperating Industry, Agency, Non-Profit, or University Organization(s)

Otolaryngology Department, U.C. Davis Medical Center

VI. Executive Summary

A lateral-flow assay (LFA) for β-2-Transferrin (β2T) was developed (Figure 1). In the assay, the target protein binds to the capture antibodies at the test line producing a visible readout. All antibody deposition was completed by reagent applicator to maximize signal localization. We found a direct LFA β2T concentration of 0.16 mg/mL produced a visible positive line in an assay that involved the direct deposition of β-2-Transferrin onto the nitrocellulose-based lateral flow device. This indicated the successful capture of antibody and colorimetric indicator. No control lines were utilized in preliminary devices.

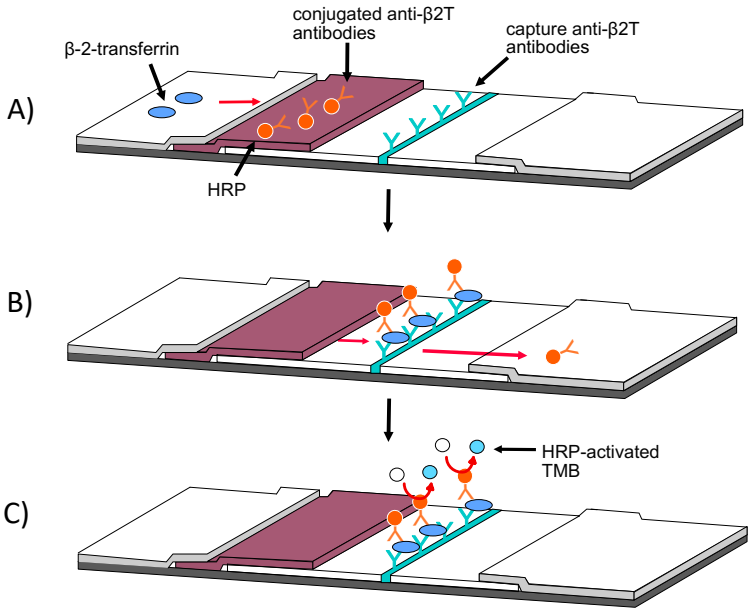

Figure 1. (A) Beta-2-transferrin is deposited on sample pad. (B) β2T begins to flow via capillary action. The analyte first reaches the conjugate pad and binds to anti-β2T antibodies which are bound to the enzyme horseradish peroxidase (HRP). The β2T continues to flow across the nitrocellulose and forms a sandwich with capture antibodies. Unbound anti- β2T antibodies continue to flow towards the absorbent pad. (C) After a washing step, TMB is used to visualize the presence of HRP.

LFAs were initially designed for readout by antibody conjugation to streptavidin bound gold nanoparticles, a common technique used in many commercial lateral flow assays, including the at-home pregnancy test. However, in the limited time allotted with a capillary flow-driven device, the conjugation of the anti-β2T detection antibody to the streptavidin bound gold nanoparticles was not successful. After multiple tests confirmed the same result, the LFAs were transitioned to visualization by HRP tagged avidin. With HRP-avidin tagged LFAs, the readout produced a positive signal, but the readout was not well-localized to a single line as was initially intended due to the need for a colorimetric indicator (TMB). Direct β2T assay results demonstrated the ability to bind β-2-transferrin to HRP-conjugated antibodies and produce a qualitative readout (Figure 2). This result can be interpreted without the need for expensive in-lab equipment.

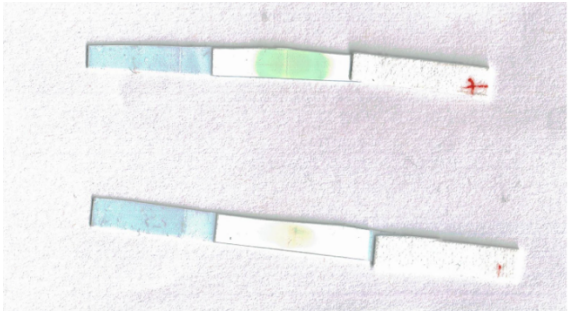

Figure 2. Positive (top devices) and negative (bottom device) results for presence of β-2-transferrin in the lateral flow assay. TMB gives a green-blue color when activated by the presence of horseradish peroxidase enzyme.

Later experiments used *in-vitro* ELISAs to analyze the ability of β2T to bind in a sandwich with anti-β2T and more general anti-transferrin antibodies. These preliminary assays worked towards the eventual goal of using β2T as a capture analyte on a flow-through assay. Further work on this project will involve optimization of a β2T sandwich lateral or flow-through assay for clinical use.

VII. Major Accomplishments

(1) Identified beta-2-transferrin as the optimal CSF-enriched protein for use as the analyte of interest.

(2) Determined the device detection thresholds and minimum volume of sample required for a variety of assay types including lateral flow assays and ELISAs.

(3) Examined the effect of another protein present in other bodily fluids, beta-1-transferrin, on assay functionality.

VIII. Expenditure of Funds

Baker/Koob funding was used primarily for purchase of reagents. Ordered materials were as follows: HyTest Transferrin Antibodies ($694), NOVUS Transferrin-HRP Antibodies ($374), Nanocs Amine Beads ($340), Anti-Rab5 Antibodies ($417), Human Rab 14 protein ($285), and Thermo Scientific Human Serum ($107). An additional $200 was allocated to taxes and shipping fees.

IX. Impact on Student Learning

This project allowed for interdisciplinary learning between the labs of Dr. Nathaniel Martinez and Dr. Andres Martinez in their respective departments of Biological Sciences and Chemistry. Dr. Andres Martinez's lab was able to provide much-needed expertise on evaporative properties in microfluidic channels which helped with the initial design of the lateral flow assay. The lab also helped with work on paper-based ELISAs which were essential to intermediate optimization steps in this project. Dr. Nathaniel Martinez's lab was able to choose reagents and analyte concentrations using *in-vitro* ELISAs and paper-based devices. The three students on this project were able to learn more not only about their respective parts of the project, but also able to gain valuable knowledge about the other department involved.