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:

Development of a Rapid Diagnostic Test for Cerebrospinal Fluid Leaks

Proposal Author: Megan Mitchell **Cal Poly Email: mmitch26@calpoly.edu**

Student ID: 012501914 **Dept: Biological Sciences**

Signature (Optional): Megan Mitchell

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
Brandon Strong	E. B. S.	ebstrang@calpoly.edu	Biological Sciences
Emily Christensen	Emily C.	echris04@calpoly.edu	Chemistry

Anticipated Start Date: Jan. 2019 **Anticipated End Date:** Jan. 2020

Total Funds Requested: $ 2500

Faculty Advisor: Andres Martinez **Department:** Chemistry

Faculty Advisor email: awmartin@calpoly.edu **Telephone:** (805)756-2744

Signature of Faculty Advisor: A. Martinez **Date:** 11/1/18

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Development of a Rapid Diagnostic Test for Cerebrospinal Fluid Leaks

Megan L. Mitchell, E. Brandon Strong, Emily Christensen, Andres Martinez PhD

ABSTRACT: Cerebrospinal fluid (CSF) leaks can place patients at significant risk of intracranial infection, including bacterial meningitis. The two most common causes of this condition are head trauma and post-surgical complication. Timely and accurate diagnosis is essential to minimizing infection risk and to facilitate treatment planning. Current diagnostic techniques include physical examination, radiological evaluation, and biomolecular assays. Physical examination is unreliable as CSF leaks can often be confused with other sinonasal disorders, and radiological evaluation often fails to detect minor leaks. Therefore, the current gold standard of CSF leak diagnosis is via biomolecular assays. However, the current tests are both time consuming (3-5 days) and expensive ($250). Alternatively, lateral flow immunoassays (LFAs) have been commonly utilized as a binary diagnostic technique for more than two decades (i.e., pregnancy test). LFAs possess many inherent advantages, including: ease of use, cost-effectiveness (~<$5), rapid results (<10 minutes), and the ability to operate without supporting equipment. The purpose of this investigation is to develop an LFA for a CSF-enriched protein. LFAs will be fabricated from a combination of paper types (glass fiber, nitrocellulose, etc.). Antibodies will be adhered to the assay via nonspecific adsorption and will allow for the immunoselection of a CSF-enriched analyte of interest. Binary detection will be confirmed using a visible molecular tag. Devices will first be tested with purified proteins, followed by pure single-donor CSF, and finally, heterogeneous mixtures of CSF with bodily fluid contaminants. This device could become an important diagnostic tool in both clinics and emergency departments globally.

OBJECTIVE(S): The primary purpose of cerebrospinal fluid (CSF) is to buffer and support the central nervous system (CNS). Under normal circumstances, the CSF and CNS are enclosed, which makes it difficult for pathogens (i.e., bacteria) to traverse. However, leaks from the cranium to the sinuses (i.e., posttraumatic) can place patients at significant risk of bacterial meningitis, which has an estimated 6-35% mortality rate.[1] While CSF leaks can occur in multiple locations on the head, the sinuses are most susceptible[2], and will therefore be the primary focus of this investigation.

Timely and accurate diagnosis of CSF leaks is important to minimize the risk of meningitis and facilitate treatment planning. Currently, there are three general methods of CSF leak diagnosis: physical examination, biomolecular assays, and radiological evaluation. Making a diagnosis based purely on physical examination can be challenging, irrespective of circumstance or cause, and can also often be confused with other sinonasal disorders.[3,4]

When CSF leakage is suspected following physical examination, CSF rhinorrhea (a.k.a., leakage through the nose) is crudely evaluated by a 'halo test', whereby nasal drainage is dropped onto filter paper.[4] If CSF is present, it will diffuse faster than blood and produce a visible 'halo' around the blood sample. While the test is rapid and cheap, it is non-specific as it only detects

the presence of a fluid less viscous than blood.[4] In order to make a more definitive diagnosis, a β2-transferrin (β2T) assay can be performed. β2T protein is specific to the CSF, and this assay is the current gold standard of CSF leak diagnosis.[5] However, there are significant limitations of this method, including cost (~$250) and delayed return of results (~3-5 days).[4] Finally, radiologic evaluation (i.e., high resolution computed tomography) can be used to determine the anatomical location of skull base defects. However, the sensitivity of this technique for detecting CSF leaks is poor.[5,6]

A rapid and cost-effective diagnostic assay is needed to provide clinically relevant data to physicians as they determine the extent of posttraumatic injuries. Lateral flow immunoassays (LFAs) are often viewed as the ideal binary diagnostic technique in point-of-care settings due to the following inherent characteristics: cost-effectiveness (<~$5), ease of storage (vacuum sealed, room temperature), extended shelf life (>1 year), ease of use (no technical expertise required), relatively low sample volume requirements (<120 µL), high specificity (immunoassay), rapid provision of results (<10 minutes), portability (<1 g), and ability to operate without supporting equipment or sources of power.[7] In this assay, the analyte is 'sandwiched' between an immobilized capture antibody and a tagged primary antibody. These tags allow for direct visualization. An overview of the LFA components and mechanism of detection is depicted and explained in Figure 1, with β2T protein used as the example analyte of interest. The purpose of this investigation is to develop an LFA for a CSF-enriched protein (i.e., β2T). The specific objectives of this proposal are as follows:

1) Determine the optimal CSF-enriched protein to use as the analyte of interest.
2) Determine the optimal LFA components for the application of whole blood samples.
3) Determine device detection thresholds and minimum volume of sample required.
4) Examine the effect of contaminating bodily fluids on assay functionality.

METHODOLOGY: Beta 2 transferrin (β2T) protein will first be explored as the analyte of interest in our lateral flow immunoassay (LFA) due to its use in current biomolecular assays.[4,5] The majority of reagents for this project are available in kits intended for enzyme-linked immunosorbent assays (ELISAs) from MyBioSource. The kits include β2-transferrin standard samples and horseradish-peroxidase (HRP) tagged antibodies specific to the standard. HRP is a colorimetric enzyme that allows for the visualization of our test results.

Paper materials specifically intended for use in lateral flow immunoassays (LFAs) (Figure 1A) can be purchased through GE Whatman. LFAs will be fabricated using Whatman Nitrocellulose Membrane Cards (FF270) (0.4 x 4 cm) with additional adhesive zones for the application of the sample, conjugate release, and absorbent pads (Figure 1A). Whatman CF4 (0.8 x 4 cm) will be used for the absorbent pad, and Whatman Standard 17 glass fiber paper for the conjugate release (0.4 x 0.8 cm) and sample (0.4 x 1.2 cm) pads (Figure 1A).

Capture antibodies (Figure 1B) will be adhered to the nitrocellulose membrane via nonspecific adsorption and applied via pipette. After applying both sets of capture antibodies (Figure 1B), the membrane will be blocked with bovine serum albumin (BSA) to prevent unwanted nonspecific adsorption of analytes or tagged antibodies. Tagged antibodies will be desiccated onto the conjugate release pad prior to LFA construction (Figure 1). All LFA components will be cut with scissors. An adhesive backing card will be used to keep all components in place. This test strip will then be placed in a plastic cassette prior to testing.

Reagent concentrations will be adjusted until optimal visual results are achieved. Should β2T prove unsuccessful in our assay, additional CSF-specific analytes (i.e., neuron-specific enolase, beta trace protein) will be explored and custom antibodies for these analytes will be purchased.

Following analyte selection, devices will first be tested with purified proteins, followed by commercially-available pure single-donor CSF, and finally heterogenous mixtures of CSF with whole blood (to simulate posttraumatic CSF rhinorrhea). Incorporation of blood separators in place of the sample pad (Figure 1A) will be explored. Blood separators are a method of size exclusion that keep whole blood cells from interfering with the assay.

Following the optimization of device components, minimum analyte detection thresholds and minimum sample fluid volumes will be determined. Finally, adequate assay functionality in the presence of contaminating body fluids (i.e., commercially-available blood, saliva, sweat) will be tested.

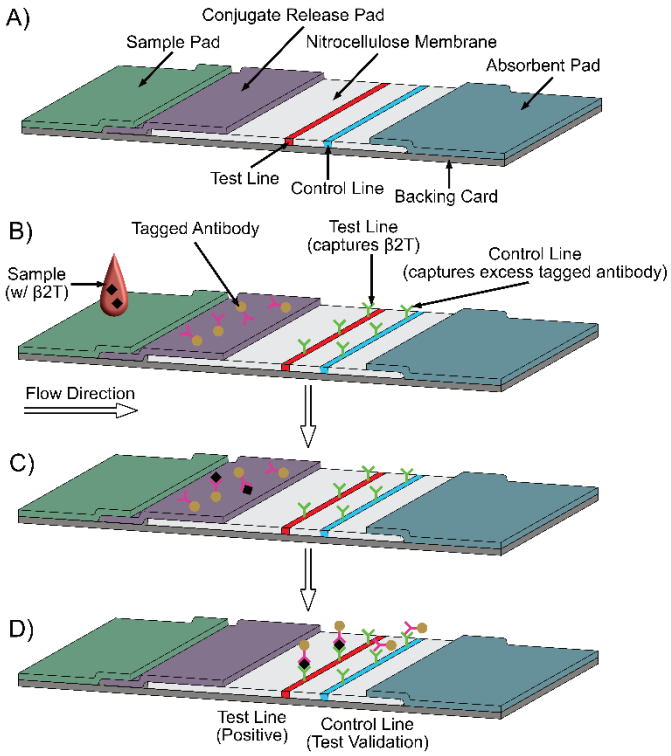

Figure 1. *Lateral flow immunoassay (LFA) overview. A) LFA components. B) Diagrammatic illustration of reagents prior to sample application. The conjugate release pad holds mobile tagged antibody (Ab), capture Ab are immobilized in lines on the nitrocellulose membrane, and lateral fluid wicking is promoted by the absorbent pad. C) β2T protein first binds to tagged Ab after wicking through the sample pad. D) The test line will capture β2T (as well as attached tagged Ab), while the control line will capture any unbound tagged Ab. Tags allow for test visualization. Two lines indicate a positive test. β2T=β2-transferrin protein.*

TIMELINE

January – March 2019: Optimization of reagent concentrations for best-visualized LFA readout.

April – June 2019: Determination of optimal CSF-enriched protein to use as the analyte of interest in this project and optimization of LFA components for the application of whole blood samples.

July – September 2019: Determination of device detection thresholds for β2-transferrin and antibody as well as minimum volume of sample required for detection.

October 2019 – January 2020: Examination of effect of contaminating bodily fluids, such as blood and mucus, on assay functionality.

FINAL PRODUCTS & DESSEMINATION: The final product of this project will be a fully functional device for rapid and specific detection of cerebrospinal fluid leakage. We hope to publish our findings in a journal such as *Lab-on-a-Chip* and to present the findings at PittCon in early 2020.

BUDGET JUSTIFICATION: Custom antibodies and/or ELISA kits from MyBioSource (contain antibodies and purified standard) ($2000), Whatman paper materials for LFA fabrication ($660), commercially available single-donor CSF ($200) and bodily fluids ($200), airfare to present at a conference ($500).

REFERENCES

1. Durand ML et al. Acute Bacterial Meningitis in Adults--A Review of 493 Episodes. *New England Journal of Medicine.* 1993;328(1):21-28.
2. Bachmann-Harildstad G. Diagnostic values of beta-2 transferrin and beta-trace protein as markers for cerebrospinal fluid fistula. *Rhinology.* 2008;46(2):82-85.
3. Le C et al. Management of Anterior Skull Base Cerebrospinal Fluid Leaks. *Journal of Neurological Surgery Part B: Skull Base.* 2016;77(05):404-411.
4. Strong EB et al. Frontal sinus fractures: A surgical management paradigm. *Otorinolaringologia.* 2017;67(1):10-25.
5. Warnecke A et al. Diagnostic relevance of β2-Transferrin for the detection of cerebrospinal fluid fistulas. *Archives of Otolaryngology–Head & Neck Surgery.* 2004;130(10):1178-1184.
6. Phang SY et al. Management of CSF leak in base of skull fractures in adults. *British journal of neurosurgery.* 2016;30(6):596-604.
7. Posthuma-Trumpie GA, et al. Lateral flow (immuno) assay: its strengths, weaknesses, opportunities and threats. A literature survey. *Analytical and bioanalytical chemistry.* 2009;393(2):569-582.

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Proposal Budget

Student Applicant(s):	Megan Mitchell
Faculty Advisor:	Andres Martinez
Project Title:	**Requested Endowment Funding**
Travel *subtotal*	$500
Travel: In-state	$0
Travel: Out-of-state	$500
Travel: International	$0
Operating Expenses *subtotal*	$2,860
Non-computer Supplies & Materials	$2,860
Computer Supplies & Materials	$0
Software/Software Licenses	$0
Printing/Duplication	$0
Postage/Shipping	$0
Registration	$0
Membership Dues & Subscriptions	$0
Multimedia Services	$0
Advertising	$0
Journal Publication Costs	$0
Contractual Services *subtotal*	$0
Contracted Services	$0
Equipment Rental/Lease Agreements	$0
Service/Maintenance Agreements	$0
TOTAL	$3,360

CAL POLY

California Polytechnic State University
San Luis Obispo, CA 93407

November 5, 2018

RE: Letter of support for Baker and Koob Endowment proposal

Project Title: Development of a Rapid Diagnostic Test for Cerebrospinal Fluid Leaks

Student Applicants: Megan Mitchell (BIO), Emily Christensen (CHEM), and Brandon Strong (BIO).

Dear Baker and Koob Endowment Proposal Selection Committee,

With this letter, I enthusiastically support Megan Mitchell, Emily Christensen and Brandon Strong's proposal for a Baker and Koob Endowment award. Their proposal is focused on the development of a rapid diagnostic test for cerebrospinal fluid (CSF) leaks. This type of device could have an immediate positive impact by helping doctors detect CSF leaks in real time, rather than having to rely on inconclusive simple tests or wait for days for the results of more reliable tests. What I find most exciting about this proposal is that the students have found a niche application (detection of CSF leaks) for a well-established technology (lateral-flow assays), and it stands to reason that the students will have a high chance of success with their project since the reagents and materials they are planning to use for their test are commercially available. Furthermore, Brandon Strong has personal connections with faculty in the Otolaryngology Department at U.C. Davis and will be able to obtain valuable advice and feedback on their project. These connections also leave open the possibility for continuing this project beyond the initial development of the strip and testing the device in hospitals (this is beyond the scope of the current application, but the potential exists for using the results of the initial work funded by this Baker Koob award to apply for further funding).

The proposal was prepared entirely by the student applicants. Their budget is targeted toward purchasing the reagents and materials they will need to develop their proposed test. I have expertise in the development of rapid diagnostic tests and will be able to help guide the project. The students will also have access to my research laboratory to conduct the work. This space is already equipped with everything the students will need to carry out the project. The students also have a clear plan for disseminating the results of the work in the form of a journal article publication and a presentation at the premier conference on analytical chemistry and diagnostics (PITTCON 2020).

Please do not hesitate to contact me if you have any additional questions about the proposal.

Best regards,

Andres W. Martinez
Assistant Professor
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