I. Point-of-Care Diagnostic Device for the Quantitative Analysis of Human Estradiol at Low-Picomolar Concentrations

E. Brandon Strong, Zachary B. Hoffman, Brittany A. Lore

II. Abstract:

A fundamental issue in healthcare is the procurement of dependable treatment, irrespective of economic means. The purpose of this investigation is to develop an inexpensive, reliable, and portable point-of-care diagnostic device for the quantitative analysis of 17-β-estradiol at low-picomolar concentrations. This assay will provide healthcare professionals with accurate data to ensure proper diagnosis and treatment. The prototype will be comprised of a hybrid combination of *paper-based microfluidics* and *in-series competitive heterogeneous immunoassays*.

III. Introduction:

A central challenge of today's world is the provision of reliable and cost-effective medical care. Paper-based microfluidics are emerging as inexpensive, reliable, and portable assays capable of providing healthcare professionals with real-time data to ensure accurate diagnosis and treatment.[1] Furthermore, these devices offer economical and efficient alternatives to current laboratory methods, such as phlebotomy.[2,3] Accurate monitoring of female reproductive hormones is of paramount importance for the diagnosis and treatment of amenorrhea, hypoestrogenicity, menopause, menstrual dysfunction, and in-vitro fertilization (IVF).[4] In-vitro fertilization, for example, requires phlebotomy for 8-10 consecutive days. A paper-based diagnostic device could eliminate the need for phlebotomy, thus reducing healthcare costs while maintaining diagnostic accuracy.[7]

While development of a prototype estradiol detection device would significantly impact fertility treatment and reproductive health, subsequent paper-based assays for a myriad of alternative serum and salivary biomarkers could exponentially impact health care as a whole.

IV. Objective:

The purpose of this project is to develop a cost-effective, home-use, diagnostic device capable of quantifying human estradiol at low-picomolar concentrations.

V. Methodology:

To provide a more convenient and cost-effective method of human estradiol monitoring, a real time point-of-care quantitative assay will be developed. This prototype will detect 17-β-estradiol present in salivary samples at low picomolar concentrations.[3,5]

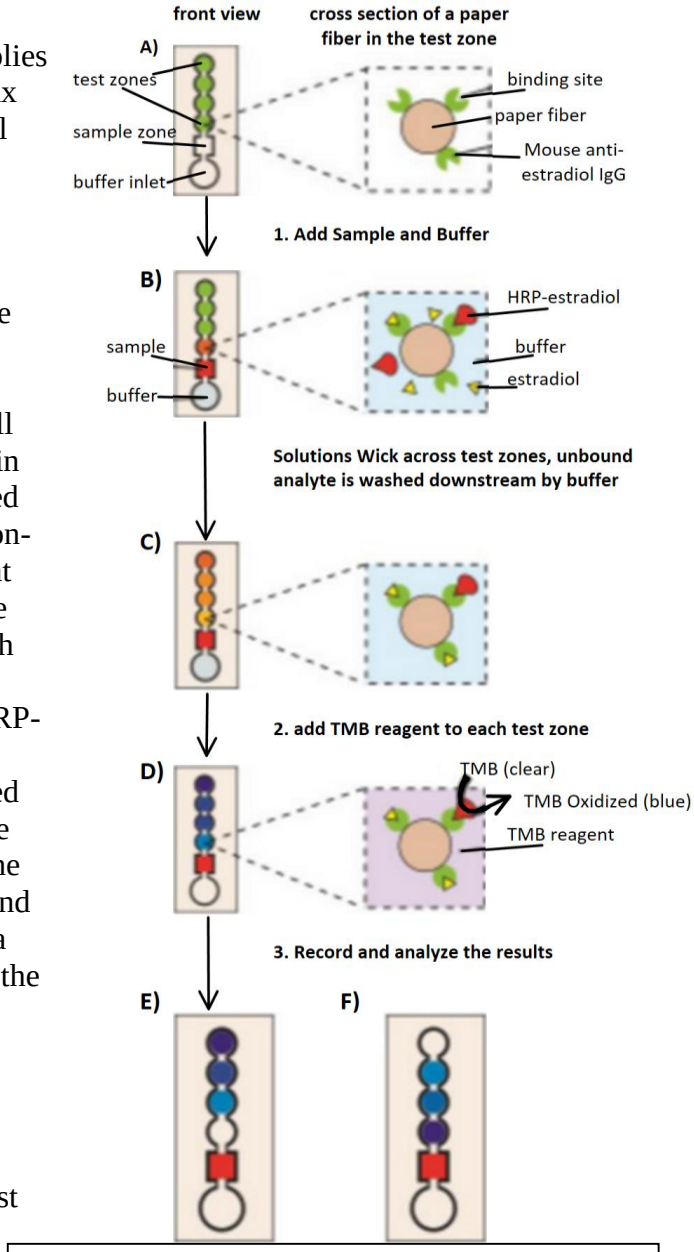
A hybrid combination of ***paper-based microfluidics*** and ***in-series competitive heterogeneous immunoassays*** will be the basis of this project. Paper-based microfluidic devices provide an ideal platform as they are inexpensive, portable (mass<1g), easy to use (capillary action used to wick fluids), require only a small sample (~15μL), and can provide real-time quantitative results.[6] With *competitive heterogeneous assays*, labeled molecules compete for binding sites with the analyte of interest.[8] A positive, quantitative result is denoted by a lack of the labeled molecule in the binding site following competition. The combination of these two modalities will yield a new mechanism for distinguishing analytes on paper, thus expanding the scope of paper-based assays.

The project will have three stages: 1) development of a fabrication method for the prototype, 2) design of a working protocol for the assay, and 3) optimization of storage conditions for the working reagents.

Fabrication Method: The paper-based devices (~12cm^2) will be fabricated on Whatman No. 1 CHR paper utilizing a method known as wax printing.[9,10] Wax printing produces a

hydrophilic channel surrounded by hydrophobic barriers. After the printer applies a layer of wax, heat is used to allow the wax to transverse the paper. This prototype will include a buffer inlet, a sample zone, and multiple in-series test zones.

Protocol: Given that the analyte of interest is estradiol, the prototype will be designed to identify commercially available HRP (horseradish peroxidase)-conjugated estradiol. Known amounts of mouse monoclonal anti-estradiol IgG antibody will be immobilized on the in-series test zones in order to bind estradiol from solutions placed in the sample zone (Figure 1A,B). Both non-specific adsorption and covalent attachment will be tested as means of immobilizing the antibodies on paper.[8] Artificial saliva[11] with known concentrations of estradiol will be premixed with known concentrations of HRP-estradiol before being placed in the sample zone (Figure 1B). Buffer will then be added to the buffer inlet allowing the sample to be wicked through the in-series test zones. The mouse anti-estradiol IgG antibodies will bind the sample estradiol and HRP-estradiol in a ratio proportional to the amount of each in the mixed sample. All unbound estradiol and HRP-estradiol will be washed downstream into the final test zone (Figure 1B,C). Following the wicking process, TMB substrate reagent (3,3',5,5'-tetramethybezidine) will be added to the test zones for signal amplification. TMB is a substrate for horseradish peroxidase (HRP) producing a blue reaction product in the presence of HRP (Figure 1D). We predict that quantifiable "fingerprints" demonstrating a color change from white to blue can be obtained (Figure 1E,F). These fingerprints will represent varying concentrations of analyte in the sample being tested.



The size, shape, and number of test zones will be optimized, detection thresholds will be determined, and the effect of alternative hormone (ie. testosterone and estrogen) interference will be tested. Time-lapse photos will be captured by a digital camera throughout the testing process.

Storage Conditions: Optimal storage conditions for utilized reagents will also need to be determined. Most proteins and antibodies denature when dried on paper, therefore the effects of trehalose, a known protein stabilizer[12], will be investigated to determine the shelf life for devices stored at room temperature.

References:

[1]Yetisen, A.K., et al. *Lab Chip.* 2013, 13, 2210-51.
[2]Abnova, Estradiol (Human) ELISA kit, vs. 03.
[3]Lu, Y., et al. *Fertil Steril.* 1999, 71, 863-8.
[4]Phelps, J.Y., et al. *Fertil Steril.* 1998, 69, 1015-9.
[5]Gandara, B.K., et al. *Ann N Y Acad Sci.* 2007, 1098, 446-50.
[6]Martinez, A.W., et al. *Anal. Chem.* 2010, 82, 3-10.
[7]Connolly, M.P., et al. *Human Reproductive Update.* 2010, 100, 1-11.
[8]Hinds, J.A., et al. *Clin. Chem.* 1984, 30, 1174-78.
[9]Lu, Y., et al. *Electrophoresis.* 2009, 30, 1497-1500.
[10]Carrilho, E., et al. *Anal. Chem.* 2009, 81, 7091-95.
[11]Gibson, J., et al. Biotechnology and Genetic Engineering Reviews. 1994, 12, 39-61.
[12]Jain, N.K., Roy, I., *Protein Science.* 2009, 18(1), 24-36.

VI. Timeline:

Milestone:	**Date:**
Develop fabrication method for prototype	September, 2016
Develop working protocol for assay	March, 2017
Optimize storage conditions	June, 2017

VII. Final Products and Dissemination:

Given the novelty and approach of this proposal, we envision the following outcomes from this project:

1. Published data in a peer reviewed journal such as: *Angewandte Chemie* or *Lab on a Chip (RSC)*
2. We expect to present our work at international microfluidics conferences such as: *MicroTAS, Dublin, Ireland 2016*
3. We expect to present our work at local undergraduate scientific conferences such as the *COSAM Student Research Conference*
4. Senior Project: This research project will serve as material for Brandon Strong, Brittany Ann Lore & Zachary Hoffman's senior project

VIII. Budget Justification:

1. **Equipment**: The Martinez laboratory will provide the necessary equipment for this project. The following equipment will be purchased: Digital camera for time-lapse imaging of device ($400)
2. **Materials & Supplies**: Lipofectamine 2000 Transfection Reagent (1.5ml_Life Technologies_$528), Anti-estradiol (100ul_Abcam_$385), Anti-biotin (500ul_Abcam_$389), Streptavidin-Dynabeads (4 ml_ThermoScientific_$952), Nitrocellulose Membrane (Bio Rad_$322), Whatmann Paper #1 (500pages_Sigma_$840.55)
3. **Computer Supplies**: Time-Lapse Imaging of the devices will require large amounts of storage for all of the images captured during the above project. A 1TB portable external hard drive (Lacie 1TB rugged Hard Drive $175) will be required for proper safe-storage of files and data.
4. **Journal Publication**: Given the novelty of this project, we expect our research to be published in a peer-reviewed journal. Based on current publication fees (*Angewandte Chemie*), a scientific paper of six pages in length and three colored pictures will total $979.

Warren J. Baker Endowment
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Robert D. Koob Endowment *for Student Success*

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PROPOSAL BUDGET

Student Applicant(s):	
Faculty Advisor: **Nathaniel W. Martinez**	
Project Title:	**Requested Endowment Funding**
Travel *subtotal*	$0
Travel: In-state	$N/A
Travel: Out-of-state	$N/A
Travel: International	$ N/A
Operating Expenses *subtotal*	$ 5000
Non-computer Supplies & Materials	$3836
Computer Supplies & Materials	$175
Software/Software Licenses	$
Printing/Duplication	$
Postage/Shipping	$
Registration	$
Membership Dues & Subscriptions	$
Multimedia Services	$
Advertising	$
Journal Publication Costs	$979
Contractual Services *subtotal*	$0
Contracted Services	$N/A
Equipment Rental/Lease Agreements	$N/A
Service/Maintenance Agreements	$N/A
TOTAL	$5000

CAL POLY
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January 30th, 2016

Re: Letter of Support for Brandon Strong, Brittany Ann Lore & Zachary Hoffman (Baker & Koob Endowment)

Dear Endowment Committee,

With this letter I enthusiastically support Mr. Brandon Strong, Ms. Britttany Ann Lore and Mr. Zachary Hoffman in their pursuit for funding of the proposed project "Point-of-care diagnostic device for the quantitative analysis of human estradiol at low picomolar concentrations". I have had the pleasure of working with each of these students in the laboratory and in the classroom setting, and I have no reservations that they have the knowledge and enthusiasm to achieve the specific aims of this project.

The field of paper-based microfluidic devices remains in its infancy and the possibilities for developing inexpensive, quantitative, at point-of-use devices are numerous, making this project a fantastic starting point for our students. The goal of this project is to develop a quantitative test for monitoring picomolar levels of human estradiol. We hope to optimize this device for use in medical settings, including use for in-vitro-fertilization.

During the course of this proposed project, I will serve as an PI and mentor for these three students. I will ensure that proper techniques are learned and performed, while delivering sound and meaningful scientific data. My laboratory will be at these student's full disposal, as will be my time and input. I have no doubt that if supported with this endowment, not only will the impact of these funds reach these three students, but it will continue to impact any new student joining this project and my laboratory.

Please do not hesitate in contacting me should you have any questions regarding Brandon, Brittany and Zachary's candidacy for this wonderful award.

Best regards,

Nathaniel W. Martinez M.D., Ph.D.
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