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CERVIS: Cervical Cancer Early Response Visual Identification System

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ENTITLED

CERVIS: CERVICAL CANCER EARLY RESPONSE VISUAL IDENTIFICATION SYSTEM

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE

IN

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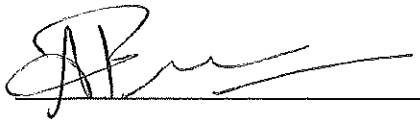

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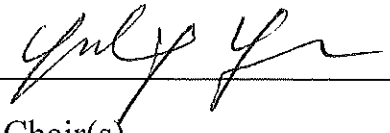

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CERVIS: CERVICAL CANCER EARLY RESPONSE VISUAL IDENTIFICATION SYSTEM

By

Evangelia Bouzos, Ivy Fernandes, and Marina Predovic

SENIOR DESIGN PROJECT REPORT

Submitted to
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in Partial Fulfillment of the Requirements
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Abstract

Our goal is to make a positive impact in the cervical cancer diagnostic space through the development of an accurate, cost effective solution that enables women in low resource settings to test for cervical cancer on a frugal and effective platform. In developed countries, we rely on regular preventive care, such as pap smears, to identify any cellular abnormalities that may indicate the disease state. However, due to the high cost and laboratory requirements of this procedure, women in low resource settings typically do not have access to this procedure. Since they are not regularly screened and often have little knowledge of cervical cancer, they are unaware of symptoms and physiological changes that mark the progression from human papillomavirus (HPV) infection to cervical cancer. Because of the seemingly benign symptoms of the disease, cervical cancer is the third most common form of cancer in women and the second leading cause of cancer related death in women worldwide. There are alternative methods available to detect the presence of high grade lesions in the cervix, but these methods are invasive and difficult to accurately interpret without the presence of a medical professional. Therefore, we are attempting to create a low cost, non-invasive, cancer-specific detection system based on urinalysis biomarker assays. We hope to launch our device in conjunction with an educational program that focuses on women's health, HPV, cervical cancer, and basic instructions for usage and interpretation of our product.

Acknowledgements

We thank our advisors, Dr. Prashanth Asuri in the Bioengineering Department and Dr. Michele Parker in the Public Health Department for their unwavering support and guidance throughout this process. We are thankful for the use of lab space and materials that made this project possible, but most importantly we are thankful for their presence. They shared in our triumphs and kept us motivated during the struggles. They pushed us to achieve more and apply to a conference that we would never have known about in the first place, let alone get accepted to. We are grateful to have them both as mentors.

We are thankful for our collaboration with Engineering World Health which gave us access to our three public health students, Lea Daran, Christina Kraus and Alyssa Miawotoe. They were essential in helping us choose our target population and understanding the impact our device could have in low-resource settings.

We thank Allan Beaz Morales, from the Frugal Innovation Hub, Julia Kramer, the developer of Visualize, and Judith Smith, the founder of the Buturi Project, for their guidance. They gave us a deeper understanding our target population and provided key advise on how to deploy our device. Without their help, we would never be able to make the transition from the lab to the field.

We thank our friends and families who have listened to our victories and our struggles during this process. Their support has kept us moving forward.

We would also like to thank Santa Clara University School of Engineering for giving funding for the project and the opportunity to make a difference in the world. We are grateful for the experiences we have had that enabled us to grow as researchers, scientists, engineers, and people.

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List of Abbreviations

CC- Cervical Cancer

CIN- Cervical Intraepithelial Neoplasia

HPV- Human Papillomavirus

VIA- Visual Inspection by Acetic Acid

ELISA- Enzyme Linked Immunosorbent Assay

LFA- Lateral Flow Assay

CHAPTER 1: Introduction

1.1 Cervical Cancer in Developing Countries

Cervical cancer is the third most common form of cancer in women around the world, of which about 80% of cases occur in lower income countries. The areas primarily affected by the disease include sub-Saharan Africa, Central America, and Melanesia.[1] This imbalance is primarily due to the lack of regular preventive care or screening of precancerous lesions, an intervention that has long since been in place in the United States. Studies estimate that while cervical screening rates in developed countries meet or exceed the 50% mark for the past 5 years, only 5% of women who reside in developing countries have had access to screening for cervical dysplasia.[1] The disparity in cervical cancer rates between developed and developing countries is also due to the high cost of the newly developed vaccinations that have been deployed and established as routine care in most of the developed world. Due to the long development period of cervical cancer, which can take as long as 20 years to progress from a mere HPV infection to an invasive carcinoma, there are many opportunities to detect and treat the disease in its earliest stages.

1.2 Human Papillomavirus to Cervical Cancer

Human Papillomavirus (HPV) is one of the most common sexually transmitted infections, such that almost every sexually active individual will be infected at some point in their lives.[2] There are over 40 types of HPV that can be spread through direct sexual contact, which fall into two categories: low-risk and high-risk HPV.[3] Low-risk HPV can cause genital warts, while high-risk HPV may cause cervical cancer in rare cases. Of all the high-risk strains, HPV 16 and 18 are responsible for approximately 70% of all cervical cancer cases. These strains infect epithelial cells and can lead to cervical intraepithelial neoplasia, of which the most severe cases may progress into cervical cancer (CC).[3] CC occurs when the cells lining the cervix, the lower part of the uterus, begin to grow out of control. Most cases begin in the transformation zone, an area where the two different parts of the cervix, covered by two different types of cells, converge. When abnormalities arise from this cellular conversion, they are referred to as cervical intraepithelial neoplasias (CIN). There are three disease states associated with CIN, separated into stage 1, 2 and 3. These states describe the extent the abnormal cells have expanded through the surface layer of the cervix, as

well as treatments needed to remove the cells. CIN1 affects one-third of the thickness of the surface layer of the cervix, indicating mild changes that often do not lead to cancer. The treatment methods for CIN1 is often a medical recommendation for continued monitoring. CIN2 affects two-thirds of the surface layer, indicating moderate changes. At this stage, there is a possibility that cancer is involved. CIN3 (also known as carcinoma-in-situ) indicates more severe changes. Although it still may not specify cancer, it needs to be treated as soon as possible.[4] None of the disease states cause symptoms, which is why cervical screening is imperative. There are three main types of cervical cancers: squamous cell carcinomas, adenocarcinomas, and mixed carcinomas. These cancers often occur in women aged 20-50. The normal cells of the cervix gradually develop through stages of "pre-cancer." Although only some of the women in the pre-cancer stage will develop cervical cancer, treating all cervical pre-cancers is the safest way to ensure a lower prevalence rate.[5]

There are several measures that can be taken to prevent HPV and CC. One of these measures is practicing safe sex, particularly through the use of condoms or safety informed sexual practices. Other ways to reduce the risk are to avoid smoking and to be vaccinated. The vaccine works to prevent HPV infection, but cannot treat an already existing infection.[5] Prior vaccines have only protected against strains 16 and 18, the high-risk strains of HPV, but the newer vaccine protects against those and also two other strains that often lead to genital, anal, or oral warts.[6] Additionally, having many children or a weakened immune system, such as those with HIV/AIDS, can also increase the risk of infection with high-risk HPV.[7] Currently, there are no treatments for HPV infections; however, symptoms like genital warts, benign tumors, and cancerous changes in the cervix resulting from HPV can be treated.[3] There are several methods of treatment for the common types of CC including surgery, radiation therapy, chemotherapy, and targeted therapy. The most commonly used screening method in developed countries is the Papanicolaou (Pap) test, a procedure that collects cells from the cervix and analyzes them under a microscope to find cancer and pre-cancers.[5]

1.3 Case Study: Tanzania

Tanzania is a country of just under 54 million people, with a median age of only 17.7 years. This is the largest population of East African countries, but it also has the lowest population density,

meaning that about a third of the population lives in rural conditions. There is a significantly high fertility rate, such that each woman will typically bear between 4 and 5 children in their lifetime, with the median age of the first birth occurring at around 19.8 years. The average school life expectancy is only 8 years for both men and women, and the contraceptive prevalence rate is 38.4%.[8] With such a significant portion of the population being so young and sexually active, and the lack of education or usage of physical contraception, there comes great risk for serious sexually transmitted diseases, such as HIV and HPV. Tanzania has the 6th highest rate of incidence of cervical cancer in the world, with 54 cases per 100,000 people.[9] About 3.3% of women have HPV 16 or 18, which will likely develop into cervical cancer.[10] In addition to having such high rates of incidence, Tanzania is one of a few countries in Africa that is prioritizing improving screening methods for this disease. However, only 12 of the 21 regions in Tanzania have screening clinics and only 1 in 20 women in Tanzania are screened for cervical cancer.[11]

1.4 Review of Existing Screening Methods

1.4.1 Current and Novel Approaches

The current medical standards for screening and prevention of HPV and cervical cancer are the Pap test and the HPV vaccine. The HPV vaccine is suggested for children to prevent against new HPV infections, however, it cannot cure any existing infections. [5] Because of this, women are suggested to receive routine screening of the cervix with a Pap test. Pap smears are highly invasive, as it requires a sample to be directly taken from the cervix and must be done by a trained clinician.[5] The microscopic analysis of this sample in a lab can cost hundreds of dollars per person, which is not a viable option in countries where many people do not consistently receive basic welfare check ups.

A more specific alternative to the pap test is Roche's CINtec histology. Roche's CINtec histology test for HPV is more specific than a pap test because it uses two biomarkers: p16 and Ki-67. The test requires a biopsy of the patient to be taken and used to quantify the amount of p16 and, Ki-67 present, as well as 12 pooled hrHPB markers for HPV16 and HPV18. The test confirms the presence of high-grade cervical tissue and can confirm precancerous cervical lesions that are

generally missed by the staining that is used for pap tests. The test was recently FDA approved as a result of the Cervical Tissue Adjunctive Analysis Study.[12]

1.4.2 Current Low Cost Methods of Screening

A commonly used diagnostic method in lower-income countries is visual inspection by acetic acid (VIA). This method is a best applied in low-resource setting due to the low cost of implementation. The screening itself consists of application of acetic acid (ordinary table vinegar) to precursor lesions in the cervix that can be caused by HPV. A positive VIA test will turn these lesions white a few minutes after application. This test works quickly and provides patients with immediate results that can be coupled with treatment in the same visit. However, invasiveness, specificity, and sensitivity, are concerns that are not addressed by this screening method. A systematic review of studies about VIA indicated that specificity and sensitivity both had a large range from 65%-95%. This results in many false positives.[13] Additionally, this method is not effective for women ages 50 or older because the testing area with lesions begins to recede into the endocervical canal.[14]

Another diagnostic that is being used to limit the cost of cervical cancer screening is a modified colposcope. Scientists at Duke University have created a low cost, easily portable colposcope that can be attached to common devices like a smartphone and used by healthcare professionals. This device will impact follow up screening for those who have been diagnosed with high risk HPV or cervical cancer. However, a colposcopy is generally not the first indicator for cervical cancer nor does it address the issue of invasiveness or the need for medical professionals to analyze the images taken.[15]

1.4.3 Critiques and Drawbacks of Current Methods

From a public health perspective, the most notable drawbacks of current screening techniques and low-cost screening techniques is the invasiveness. The table in **Appendix E** outlines current diagnostics options for cervical cancer. The current diagnostic options assess are visual inspection by acetic acid, the pap test, a colposcopy, as well as Roche's CINtec Histoloy Test.

1.5 Significance of Project

As previously stated, more than 84% of cervical cancer cases occur in low resource settings, notably countries in Africa, Latin America, and the Caribbean. With Tanzania having the 6th highest rate of incidence in the world, at 54 cases per 100,000 people, [9] there is great need for a cervical cancer diagnostic compatible with the countries' cultural norms, geographic vastness, and lack of medical development. However, Tanzania is one of a few countries in Africa that is prioritizing improving screening methods for this disease, meaning that they are likely to invest in programs that would fund ventures to decrease female mortality due to cervical cancer. Currently, only 12 of the 21 regions in Tanzania have screening clinics and only 1 in 20 women in Tanzania are screened for cervical cancer.[11] There is some work being done by local agencies to increase awareness of HPV and to pilot the HPV vaccine, [16] but there is great room for improvement.

These factors represent a pathway for growth that can be achieved through the creation of this device. Our frugal detection device has the capacity to make a large impact by screening more women and launching an educational program, in conjunction, that can be deployed in all regions of Tanzania and other parts of the word. It will be non-invasive, accurate, and can be used without the need of a medical professional. Being that the progression of this cancer is highly preventable with intervention, increased rates of early detection can greatly lower the mortality of cervical cancer in countries like Tanzania.

1.6 Project Goals, Objectives, and Expected Results

1.6.1 Project Goal

Our goal is to design a cost-effective solution that enables women in developing countries to test for cervical cancer in a low cost and effective platform. By the end of the academic year, we hope to validate our assays and design an immunochromatographic lateral flow assay that will detect stage 0 and stage 1 cervical cancer biomarkers in mock urine. Currently, we would like to test for two biomarkers: the polyamine spermine, which has been shown to be significantly upregulated during cervical cancer, and the p16 protein, which is strongly expressed in high grade lesions and invasive cervical cancer.

We chose our biomarkers purposefully to specifically test for cervical cancer as opposed to just HPV infection, because of the natural self-clearing properties of most infections. The two markers together will theoretically identify the presence of cervical cancer, although there is the possibility of detecting unspecified cancer or an HPV infection without the presence of cancer; both of which would be helpful results. A positive cervical cancer test result will display: Spermine (+) and p16 (+). The first biomarker, a polyamine, has been associated in the development of cervical cancer, with a substantial increase in concentration in the serum, plasma, and urine of subjects with cancer. Physiologically, polyamines have been seen to mediate immunosuppressive effects in vitro due to their oxidation products; it is theorized that this effect explains their presence during cervical cancer.[17] The second biomarker, which is the protein product of the p16 gene, is strongly expressed during high grade lesions and invasive cervical cancer.[18] The protein concentration of p16 in urine is still undetermined due to lack of literature on the subject, but our device will act as a proof of concept onto which more research can later be done. Initially, we hoped to also test for the HPV L1 protein capsid to help distinguish an HPV infection from cervical cancer; however, the biomarker was left out of our final design as the concentration of the L1 protein is not yet known in urine.

1.6.2 Objectives

Our objectives are to make our diagnostic *easily accessible* because many our customers live in remote areas. We want to have *high true positives* and low *false negatives*, as other diagnostic methods typically have a low true positive rate. Often this requires the women to undergo more invasive measures to confirm the presence of the disease. We desire our solution to be *non-invasive*, since invasive treatments can be a deterrent both culturally and physically. We aim for our device to be *easy for patient to administer*, so they do not have to go to a health clinic that is potentially far from their house to get tested. We would like our product to be *manufactured at a low cost* in the country of implementation, to prevent customers and clinics from run out of supplies and having to wait for them to be imported. We hope to implement an *educational program* with our solution so that women can learn the signs and symptoms and get tested before the cancer metastases. We presented these objectives to scientists who worked with Ghanaian people to train them in detecting cervical cancer by visual acetic acid. They approved of our objectives and provided feedback.

To determine whether our objectives are met, we expect our product to consistently test specifically and sensitively for p16 and spermine. After validating the Enzyme Linked Immunosorbent (ELISA) assays for each of these biomarkers, we expect to be able to incorporate these assays on to a lateral flow assay (LFA), which provides the optimum form of diagnostic device for the population we are targeting due to the low-cost and easily storable properties of the assay.

1.6.3 Expected Results

We outline the results one would expect to see if they used our microfluidic urinalysis diagnostic depending on their respective health status **(Table 2)**. If the individual is infected with HPV, then there is a small possibility that they may receive a positive diagnosis from the p16 LFA, if the infection is accompanied by high risk lesions. If the diagnostic expresses a positive marker for spermine, then one can infer that it is indicative of the presence of cancer somewhere in the body. If this diagnosis is obtained it is important for the patient to seek medical attention regardless of the HPV diagnosis. Finally, if there is a positive marker on both the HPV assay and the spermine assay, the we can assume that the patient is very likely has cervical cancer.

Table 2: Expected Results from Frugal Diagnostic

Potential Health Status	No Disease	Low Risk HPV Infection	High Risk HPV Infection	General Cancer	Invasive Cervical Cancer
Spermine	-	-	-	+	+
p16	-	-	-/+	-	+

1.6.4 Alternative Plan

If we are unable to validate assays for both biomarkers, then we hope to focus on validating an LFA assay for one biomarker. This biomarker will most likely be spermine, as the concentrations of spermine in an individual who is healthy and in an individual who has cervical cancer is published. After we validate the ELISA assay, we plan on optimizing it to fit a lateral flow assay, which is the optimum diagnostic for our target population.

CHAPTER 2: Systems Level

2.1 Customer Needs

Prior to the creation of our diagnostic, we felt that reaching out to the customer was essential in order to develop a device that was relevant to their needs and compatible with the area. We began by attempting to make contact with the women and healthcare providers of Tanzania on our own; however, we quickly realized that it is easier to make contacts in our target population through our partner, the Frugal Innovation Hub. Through them, we reached out to healthcare providers, researchers, and organizations that have worked in conjunction with the women of Tanzania, or with cases of cervical cancer in comparable locations. Through this network, we were able to understand the outcomes that were most important to the target population, and tailor our device to best meet those needs.

2.1.1 Customer Interviews and Literature Search

According to IMA World Health, an organization that is working in Tanzania on the cervical cancer crisis, the barriers to cervical cancer treatment include the following: lack of supplies, lack of follow up, and lack of education. If health care workers run out of supplies, they are no longer able to test for the disease. Unfortunately, due to the lack of funding and the expansive geography of Tanzania, running out of supplies is often the case with most outreach teams. Similarly, women are unlikely to follow up and get secondary pap smears if hospitals are located far from their homes. Due to the majority rural demographics of Tanzania, trips to the nearest established medical centers are often 50-100 km away from the remote villages in which these women live. Therefore, a trip can cost a woman a full day of work, and a significant portion of their wages in order to have access to a motor vehicle; both of which are significant impairments to their motivation for access. Finally, within Tanzania there is a lack of general education about women's health that is potentially due to cultural barriers, which leads to much confusion on the nature and symptoms of female specific diseases, particularly those that are spread via sexual infection.[19]

We also reached out to Julia Kramer, a graduate student at UC Berkeley who is currently involved with research on cervical cancer in Ghana. She created a training simulator model to

assist in training midwives to detect cervical using visual inspection by acetic acid (VIA). Kramer suggested that one of the most significant issues was the lack of medical attention that the women typically receive. They often do not visit a hospital in their entire lifetime, aside from instances of severe illness or childbirth. Additionally, there is the significant issue of the promiscuity stigma that prevents conversations around sex and safe sexual practices. Therefore, she stated that the bigger challenge was in getting the women through the door, not in getting them to take the test. Finally, in response to our device Kramer stated that we may struggle with implementing the preventive testing at home due to the lack of precedent and the stigma of sexually transmitted infections; however, she suggested that the diagnostic could be deployed in village community centers where the residents receive basic health aid.

2.2 Desired Outcomes

From these resources and additional literature searches, we were able to come up with a set of outcomes and rank them on importance for the customer. The product must be/have:

1. *Easily accessible* due to the challenges a woman may experience in reaching a hospital if she lives in a rural, like most women do.
2. *High true positives and low false negatives,* as other diagnostic methods typically have a low true positive rate, often requiring the women to undergo more invasive measures to confirm the presence of the disease.
3. *Non-invasive,* as invasive treatments can be a deterrent due to both cultural stigma, religious perspective, or fear of physical harm.
4. *Easy for patient to administer* so they do not have to go to a health clinic that is potentially far from their house to get tested.
5. *Low cost manufacturing* in the country of implementation so they don't run out of supplies and there is no issue with deployment.
6. *Educational programs* that accompanies the diagnostic so that the women can learn the signs and symptoms of HPV and cervical cancer, along with the ways in which is transmitted and potential safe practices.

2.3 Outcomes In Innovation

Our outcomes led us to develop an assay that is more specific for stage 0 or stage 1 cervical cancer in urine compared to HPV or invasive cervical cancer. We hope to integrate the assay into a urine test that can be self-administered at home or in a village clinic, and be easy to read in a concrete and simple manner. The product will be manufactured in the country of implementation and include an educational program within the packaging.

2.4 Project Components

2.4.1 Biomarker Selection

Before the creation of our cervical cancer diagnostic, it was necessary to select specific and scientifically backed biomarkers that would provide a strong foundation for the device. We spent a significant portion of our research on this section, as we deemed that it would reduce the credibility of our project if the biomarker were to be non-specific or non-functional. Therefore, we came up with five steps that were necessary to accomplish before any marker could be added to our device.

The first step in this process was to confirm that the biomarker was upregulated during instances of cervical cancer. We did this by searching for panels of proteins, hormones, and antibodies that had been linked to CC in previous research. Once we found compounds that seemed to have a reliable relationship with CC, the second step consisted of looking into the urine panels of patients to confirm that this compound could be detected in urine. This was an essential step because a core outcome of our diagnostic consisted in it being noninvasive, a quality that would be negated by many other bodily fluids. If the biomarker had history of occurring in urine, then we would proceed to the third step, which was finding the concentration of the biomarker in the urine of a healthy individual versus that of a diseased individual. This step was essential to the process, and also the most difficult to surpass. Due to the fact that there is no urinary cervical cancer screening precedent, there was not a lot of literature measuring the concentrations of protein in the urine of cervical cancer patients. However, we managed to locate quite a few biomarkers that were measured in urine, and in which the difference in healthy and diseased concentrations was significant. For the fourth step, we simply looked up what the conventional testing methods were

for the biomarker, which led us to the fifth step of identifying a detection method that was compatible with the target population. This meant that the detection method had to be frugal, effective, and require minimal external aid.

2.4.2 Device Format

We chose to deploy our diagnostic on a lateral flow assay (LFA) due to the significant advantages that this method has over other assay formats, particularly in the context of deployment in Tanzania, where there is no guarantee for electricity, clean water, sterile conditions, or an experienced technician. LFA's are a low-cost diagnostic test that operates on the use of antibodies, colored particles, and several physical pad components, to give a definitive confirmation regarding the presence or absence of analyte. These tests typically have long shelf lives, and do not require refrigeration or special care, like many other diagnostic tests. Another benefit of an LFA is that it requires minimal training and no additional processing after use, which makes it well suited to act as a field based diagnostic necessary for our target population, Tanzania. Because these tests are powered only by capillary action, they do not require an energy source and provide a reading within 20 minutes of testing. Similarly, they only require a small amount of liquid sample in order to produce a result. In terms of sensitivity, LFA's have better sensitivity and specificity than other well established methods under optimal conditions; however, depending on the analyte and antibody used there may be cases of low biomolecule affinity.[20]

Although LFA's have significant advantages, there are a few drawbacks that demand the assay be designed with careful consideration of the analyte. To begin with, these tests are at most semi-quantitative, and typically only qualitative. This is due to the nature of the results, which is one line for a negative reading, and two or more lines for a positive reading. However, we consider to be beneficial to our aims as it reduces the complexity in reading the device output. Depending on who is making the LFA batch, reproducibility of the test can vary significantly because of the manual fixation of antibodies and colored particles. Depending on the specific experiment, the sample may have to be pretreated, or the nitrocellulose blocked, for optimal testing. Finally, because capillary action is the only energy involved in this assay, once the sample is applied it

can neither be sped up or slowed, and the properties of the sample may affect the speed of the reaction.

2.4.3 Educational Component

In addition to the creation of our device, we hope to launch an educational component. This could be in the form of a pamphlet that serves to educate women on sexually transmitted diseases, the progression of HPV into cervical cancer, and women's health in general. Ideally, this will increase the impact of our project by providing women with information that can be taught to others in their communities. Thereby, educating the population as a whole and eventually leading to decreased rates of incidence and mortality due to cervical cancer.

2.4.4 Project Pipeline

In order to outline the various components of our project and their relationship to each other, we developed a pipeline **(Figure 1)**. The first component is public outreach. This will involve creating an infrastructure and distribution system for basic information regarding sexually transmitted disease, HPV, cervical cancer, and the benefits of routine screening. The educational component would ideally include the creation of pamphlets outlining the disease stages and treatment options. Additionally, it would involve creating partnerships with community health workers that could provide valuable information on HPV and cervical cancer.

Within this pipeline, we hope to be able to route the users of our device to the help that they need. This would entail providing proper support and guidance for those who obtain routine screening with CERVIS depending on their result. Whether the community members test positive for HPV, cervical cancer, or test negative for both outcomes, we hope to have a clear network established that can ensure the user is able to get the support they need.

We want to emphasize the importance of follow up and continued screening for the detection and prevention of disease progression. Each screening will include a test with our CERVIS device as well as explanation of results and direction of the next steps. A negative result would result with no indication of HPV or cervical cancer and would require no additional action, aside from routine screening. If the user tested positive for HPV, more frequent routine screening would be suggested.

A positive result for CC or general cancer would result in the patient being advised to seek further medical attention to confirm the diagnosis and get treatment. We hope to connect our users to healthcare professionals in their respective countries, so that there is a clear treatment method following the diagnosis of cervical cancer.

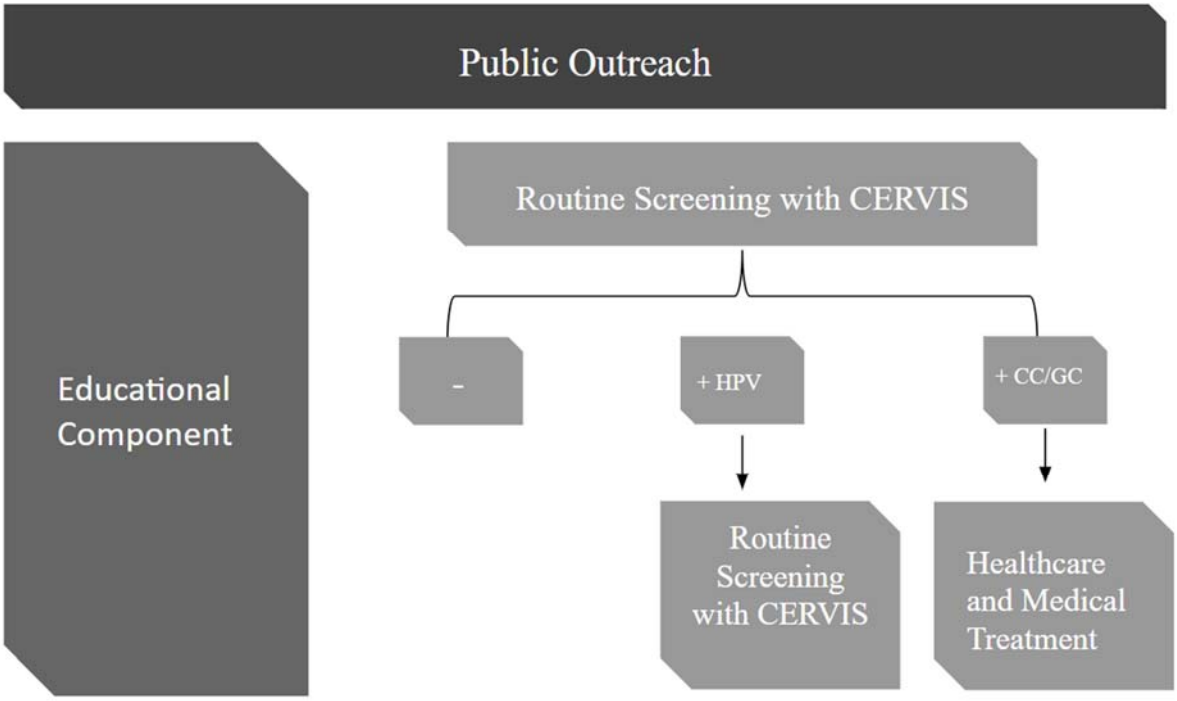


Figure 1: Project Pipeline

2.5 Team and Project Management

2.5.1 Team Management

Our team is composed of two SCU faculty members, three bioengineering students, three public health students from the Engineering World Health partnership, and two independent researchers. Our faculty members are Dr. Prashanth Asuri, who acts as our bioengineering advisor, and Dr. Michele Parker, our Engineering World Health (EWH) advisor. The public health students partnered with us through EWH are Christina Kraus, Lea Daran, and Alyssa Miawotoe. Our project originally stemmed from the work of Dr. Hernandez, a researcher at the University of Hawaii, who has since provided us with further information regarding HPV biomarkers and potential diagnostic tools. Finally, we are also in contact with Julia Kramer, a UC Berkeley Graduate student who created a training simulator model to help midwives detect cervical using acetic acid. Her

contribution to our project has been mainly in informing us of the target population and shaping our customer outcomes. Within our primary bioengineering group, Eva Bouzos acts as the primary liaison for Dr. Asuri, Ivy Fernandes interfaces with Dr. Hernandez, and Marina Predovic communicates with the public health students.

2.5.2 Budget

We requested that the Santa Clara University Undergraduate Programs help us with the cost of developing our frugal cervical cancer diagnostic. The funding we received was put towards designing an assay that validates the ability of successfully screening for certain biological markers specific to stage 0-1 cervical cancer. Due to the variety of biomarkers it was necessary to obtain multiple enzyme kits, antibodies, and reagents necessary to screen for the biomarkers and produce the desired colorimetric readout. From there, we worked on designing a method which screens for these markers in a way that is conducive to the environment in which it will be used. We requested a total of $2,800 and received $2,500. A detailed budget is located in **Appendix A.**

2.5.3 Timeline

Refer the Gantt Chart in Appendix B for a graphical representation of our time-line.

Fall Quarter

During the first few months of our project we identified the need for a low-cost cervical cancer diagnostic in developing countries. After identifying the problem (a high rate of incidence of cervical cancer in low-resource settings compared to the developed world), we worked hard to understand how this problem could best be addressed and what current methods were being used to address it. Our first task was to truly understand the disease progression. From there, we identified current screening and prevention methods including medical standards as well as methods that are currently being deployed in low-income countries.

In recognizing that each country has its own unique factors that influence the way in which a problem propagates and can be addressed, we partnered with three public health students through the Engineering World Health program, who helped us discern which country would be the most

viable place to deploy our device in. These students helped us to identify Tanzania as a viable option, and worked with us to network with healthcare professionals as well as organizations in the country. With their help as well as the help of Frugal Innovation Hub, we partnered with the Buturi Project. Through this connection as well as interviews with many other professionals and experts in the field, we were able to gain a deeper understanding of the needs in the country as well as validate that our device and it's design would be useful and accepted in the country. Finally, we performed literature searches on the biomarkers present in HPV and cervical cancer.

Winter Quarter

At the beginning of the quarter we continued our biomarker search, narrowing down the panel of potential biomarkers utilizing a checklist that ensure it was present in detectable levels in the urine as well as specific to HPV or cervical cancer. After identifying several markers, we wrote protocols and began to run experiments in the lab. Additionally, we narrowed down our biomarkers from three biomarkers to two and decided to run ELISA assays to detect the biomarkers over assays that we initially designed.

Spring Quarter

We ran and optimized ELISA assays for p16 and spermine and began the optimization process for incorporating the biomarkers into a lateral flow assay. We presented our diagnostic at the Global Health and Innovation Conference held at Yale University in April.

CHAPTER 3: Subsystem- Target Analytes

3.1 Evolution of Biomarkers

Our initial panel of biomarkers was composed of three different compounds, each of which served a specific purpose for the diagnostic. These were: the HPV protein capsid L1, which was upregulated during cases of HPV, but absent in CC; the Polyamine Spermine, which is a general cancer biomarker; and p16, a cyclin dependent kinase inhibitor that has been recommended by the World Health Organization as a biomarker of CC.[21] Therefore, by using the three biomarkers in parallel we would be able to distinguish between instances of HPV and CC. A positive CC diagnosis would consist of the presence of both Spermine and p16, but the absence of the L1 capsid **(Table 4)**.

Table 4: Initial Biomarkers and Expected Readout

	No Disease	Low Risk HPV Infection	High Risk HPV Infection	General Cancer	Cervical Cancer
Spermine	-	-	-	+	+
p16	-	-	+/-	-	+
HPV Protein Capsid L1	-	+	+	-	-

3.2 Human Papillomavirus L1 Protein Capsid

The HPV protein capsid L1 is the main protein associated with the virus, making up 90% of the total protein on the virus surface. This capsid is unique, because unlike other biomarkers it is abundant during infection, less present during the precursor lesions, but then nonexistent in carcinomas.[22] Therefore, we were going to use this biomarker to distinguish a simple HPV infection from the more serious lesions or cervical cancer. However, as we went through the

selection criteria cycle, we found several issues with the biomarker. The first issue is that although the viral load in urine is recorded, the exact concentration of the protein in urine has not been researched[23] [24] In addition, our preliminary estimation of the protein concentration indicates that it would not be detectable using the low cost and deployable methods that we had been considering.[23] [24] The only methods that were feasible for detection of the estimated concentration are not compatible with the infrastructure or resources of Tanzania.

3.3 P16

P16 is a cyclin-dependent kinase inhibitor that is upregulated in cervical cancer and precancerous cervical tissue.[25] In 2012, the College of American Pathologists (CAP) and the American Society for Colposcopy and Cervical Pathology (ASCCP) recommended the use of p16 for cervical cancer diagnosis. In 2014, the World Health Organization became the first global organization to issue a written recommendation for its use; therefore, we chose this biomarker to be our cervical cancer specific marker in our urinalysis device.[26]

In healthy cells, p16 serves as a tumor suppressor protein, as it is essential in the regulation of the cell cycle. P16 regulates the Rb pathway by inactivating the cyclin-dependent kinase that phosphorylates Rb; thereby decelerating the cell cycle and preventing unregulated cell proliferation.[28] In high risk HPV and CC, p16 is over expressed and accumulates in cells is due to the misregulation of the Rb pathway. HPV infections that are transforming into CC have an increased expression of the viral oncoprotein E7, which disrupts pRB, a key regulator in the Rb pathway. This misregulation results in the overexpression of p16 via a negative feedback loop.[25]

Although the concentrations of p16 in urine in individuals with cervical cancer are not yet published, Dr. Brenda Hernandez at the University of Hawaii is currently conducting research that verifies that p16 is secreted into the urine in the diseased state. We look forward to her paper once it is published that at we can develop our diagnostic with the concentration of p16 in mind.

3.4 Spermine

Although HPV is the known precursor for cervical cancer, because of the variability of the infection and its numerous strains, it is necessary to test for another biomarker that may be indicative of a serious disease, and therefore confirm that the patient should definitely seek medical attention. The role of polyamines in cancer is still unconfirmed, but many studies have confirmed the upregulation of these compounds in the serum, plasma, and urine of cervical cancer patients. Current research suggests that their oxidation products have immunosuppressive effects, which would explain their relevance to cancerous tissues.[17]

Three polyamines are frequently reported in literature: putrescine, spermidine, and spermine. Often these molecules are pooled to provide a larger concentration of polyamines to measure. When looking into each molecule individually and how their expression level changed due to various diseases, we settled on spermine as being our final biomarker. Unlike the other two polyamines, which are upregulated by all diseases in the urinary system, spermine is downregulated by other diseases of the urinary tract while upregulated by cervical cancer.

In the urine of a healthy subject, spermine is present at concentrations of 0.76 micromoles per gram, which converts to approximately 1979 nanograms per milliliter.[17] [28] When looking at spermine concentrations in subjects with cervical cancer, there was a calculated six fold increase in the concentration of spermine in the urine. The urine of a cervical cancer patient has 4.89 micromoles per gram of spermine, meaning that they have 12,705 nanograms per milliliter, which is 12.7 micrograms per milliliter.[17] [28] These factors made spermine an ideal biomarker to validate that a person's HPV had progressed into cervical cancer.

3.5 Final Biomarker Selection

Therefore, our final diagnostic panel consisted of two biomarkers, p16 and spermine **(Table 5)**. If a woman were to use the diagnostic and have neither appear on the lateral flow assay, then one could conclude that she most likely has no disease or a low risk HPV infection. If only p16 were to appear, then it is possible that the woman has high grade lesions, as p16 is upregulated in the case of CIN2 and CIN3. Similarly, if only spermine were to show up on the assay, then one could

conclude that the woman either has a urinary cancer, or her body is expressing some sort of dysfunction that led to the upregulation of the biomarker. In both these cases we would recommend that she see a medical professional for a general examination. Finally, if both biomarkers were present on the diagnostic then the patient would be told that they are expressing signs of cervical cancer, and would be referred to a clinic or medical practitioner who could perform a more thorough examination for cervical cancer and potentially advise them on treatment options.

Table 5: Final Biomarkers and Expected Readout

	No Disease or Low Risk HPV Infection	High Risk HPV Infection	General Cancer	Cervical Cancer
Spermine	-	-	+	+
p16	-	+/-	-	+

CHAPTER 4: Subsystem- Detection System

4.1 Evolution of Design

Initially, we planned on designing enzymatic assays for our biomarkers. Different enzymatic assay techniques were researched and protocols were written for each of the biomarkers that were identified. The enzymatic assay designed for p16 utilized a cyclin-dependent kinase colorimetric assay. This cyclin-dependent kinase ATP-ADP Glo Assay obtained from Promega involves the conversion of ATP to ADP via the p16 kinase. When the p16 kinase is active, it is able to convert ATP to ADP, creating a bright green readout. When the kinase in inhibited by staurosporine, it cannot convert ATP to ADP resulting in no colorimetric readout. Since this assay was not colorimetric, we would not be able to incorporate it into our device, as one of our constraints is a colorimetric readout **(Appendix C)**.

The polyamines enzymatic assay involved the conversion of the polyamines spermine, spermidine, and putrescine into to hydrogen peroxide via polyamine oxidases. The hydrogen peroxide formed would then react with ABTS, a colorimetric reagent, to give a blue colored output that could be visible by the eye. Though this method is widely used to quantify the amount of polyamines present in a sample, it is not specific to polyamines present in urine as there may be other natural sources of hydrogen peroxide due to biological metabolism functions.

Although these were all functional assays, they cannot be easily be incorporated into a Lateral Flow Assay (LFA), which is our final product. LFAs utilize antibodies to detect the presence of analytes; therefore, we made the transition from enzymatic assays to enzyme-linked immunosorbent assays (ELISA). ELISAs are plate-based assays that are used to detect and quantify biomolecules, such as proteins, antibodies and hormones.[29] It is a quick and simple test that produces a colored product related to the amount of analyte in the sample [30]. They are typically carried out in 96-well polystyrene plates that are designed to bind to antibodies and proteins by immobilizing the reagents. Since the reagents are immobilized on the plate, it is possible to easily separate bound analytes and unbound molecules in a sample. This ability to wash off nonspecific molecules makes ELISAs great tools for detecting and quantifying analytes in a crude product, [30] such as urine. Since our LFA will use antibodies to detect the biomarkers,

ELISA assays can be used as a proof of concept to verify that we can differentiate between the healthy and diseased state, run the assay at relevant temperatures, and detect the analyte in urine.

4.2 p16 ELISA Assay and Results

The p16 sandwich assay was purchased from MyBioSource **(Appendix D1)**. The assay comes with the p16 antibody conjugated to the bottom of the plate **(Figure 2)**. The urine sample, or buffer, is added to the plate, and if p16 is present, it will bind to the antibody. The HRP conjugated antibody is added and will bind to the other side of p16, like a sandwich. Then the HRP substrate is added to the system and results in an enzymatic reaction that turns the solution blue. The blue color is directly proportional to the amount of p16 present in the sample.

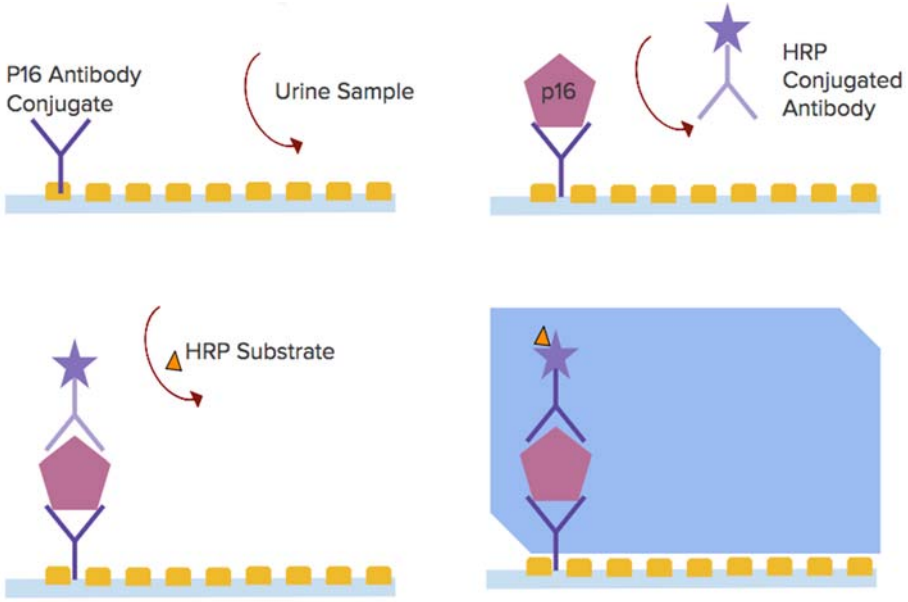


Figure 2: P16 Sandwich ELISA Assay.

4.2.1 Differentiating Between the Healthy and Diseased States

Since the levels of p16 in urine of individuals with cervical cancer are not yet published, the assay was unable to be run at concentrations of a healthy and diseased individual. However, we ran the assay at low concentrations to show that small amounts of p16 can be detected using this assay. Although the detection range was said to be 6.25 pg/mL - 200 pg/mL, we were able to comfortably detect as low as 5 pg/mL, as shown by the visible color in the wells **(Figure 3).** There was some detectable color at 2.5 pg/mL, but more optimization would be required to comfortably detect that

concentration **(Figure 3A).** Since p16 should not be in the urine of healthy individual,we were able to show that the assay is extremely sensitive and can be a valid option for differentiating between the two states.

A)

	P16 Concentration (pg/mL)			
	0.5	2.5	5	25
37° C				

B)

	P16 Concentration (pg/mL)			
	5	25	50	100
37° C				

Figure 3: Qualitative Data from Varying Concentrations of p16. (A) Image of assay when run below recommended detection range. Able to reliably see color at 25 pg/mL. **(B)** Image of assay when run at lower end of detectable range.

4.2.2 Temperature Optimization

Assay was conducted the recommended temperature of 37℃ and at room temperature. The assay was run at room temperature verify that it could be run in substandard conditions, as incubators are not common in our target population. Temperatures in Tanzania range from 18-31℃ [31] and the room temperature of the lab was around 20℃. Since the assay was still functional, as indicated by the appearance of a blue color at both temperatures, we can conclude the assay will work in Tanzania **(Figure 4)**.

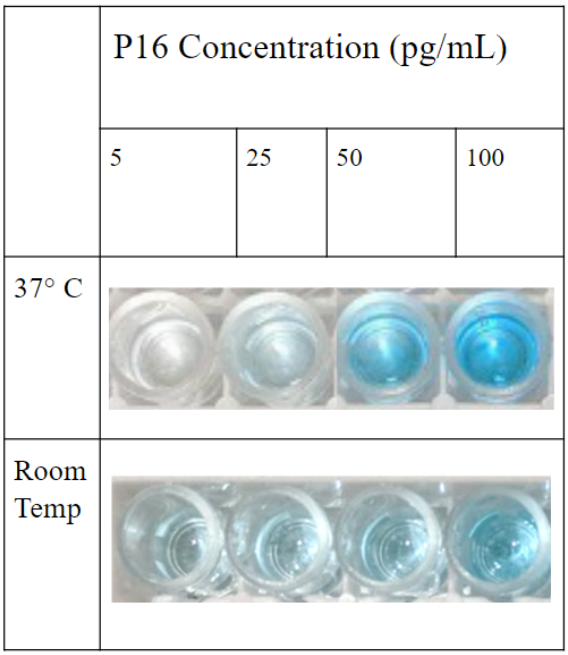


Figure 4: Qualitative p16 Data for Temperature Optimization. Image of assay run at both recommended incubation temperature as well as room temperature.

4.2.4 Fluid Optimization

Due to the non-invasive design, and subsequent urinalysis testing of the biomarkers, we had to confirm that the results of the ELISA were comparably consistent for detecting the analyte in urine, as they were in the buffer that was provided. Therefore, to determine if the assay would be functional in detecting p16 in urine, we replaced the buffer with urine at the strongest concentration of urine possible. This was done intentionally, to test the most suboptimal conditions possible. P16 was then added to the urine at varying concentrations, and placed in the wells where the ELISA reaction took place **(Figure 5)**. Qualitatively, we were able to detect p16 in urine and in buffer, as seen by the presence of the blue color at all four concentrations. Additionally, the gradient of color progressed from light to dark in both assays, confirming that urine did not impede the reaction between the analyte and the antibodies. Therefore, the p16 ELISA assay is a functional assay for the detection of p16 in urine.

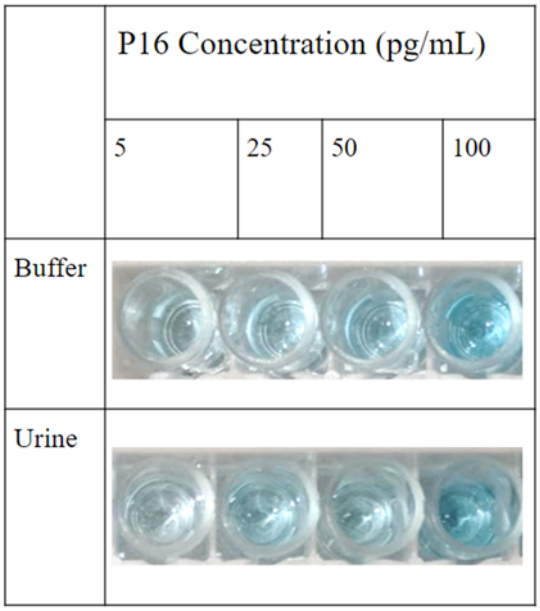


Figure 5: Qualitative p16 Data for Fluid Optimization. Images of assays run in buffer supplied with ELISA kit and of assay run with artificial urine show no distinct difference.

4.3 Spermine ELISA Assay and Results

A Spermine competitive ELISA was used to detect for polyamines in the urine. The ELISA assay obtained from MyBiosource (Catalog#MBS731360), was tested at basal concentrations as well as diseased concentrations to ensure that there is no colorimetric signal obtained in the basal conditions and only in the diseased condition **(Appendix D2)**. The ELISA works by a competitive ELISA mechanism which involves a well-plate already pre-coated with an anti-spermine antibody. That antibody binds spermine molecules that are present in the urine sample. After the sample is applied to the wells, an HRP conjugated antibody reagent is added. This reagent is highly specific to the pre-coated antibodies that do not have any spermine bound to them. Because of this, when there is no spermine in the solution, or lower levels, which can be correlated to the levels present in a healthy human, a darker blue color is produced because more colored reagents are able to bind. Conversely, when there are higher levels of spermine in the urine, as a diseased individual would, less of the HRP color reagent can bind, producing a lighter blue color.

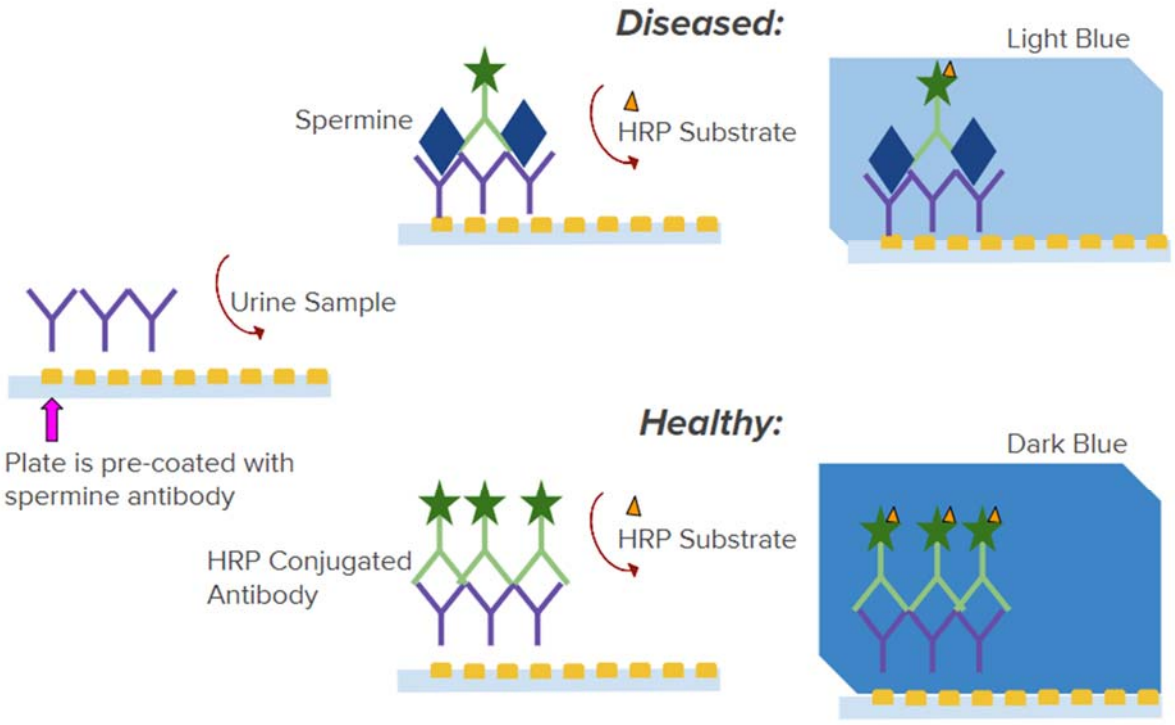


Figure 6: Spermine Competitive ELISA Assay

4.3.1 Differentiating Between the Healthy and Diseased States

Running the spermine ELISA at different concentrations of spermine enabled for the successful differentiation between the healthy and diseased states. A healthy individual has about 0.2 ng/mL of spermine in their urine and an individual with cervical cancer has 2 ng/mL. [17] Since the spermine ELISA is a competitive assay, the well with the healthy amount of spermine is a deeper blue, because there is less spermine to compete with the antibody and the well with the diseased amount of spermine is clearer in color, since there is more spermine to compete **(Figure 6)**. After running the assay and taking pictures, a plate reader was used to measure the absorbance of each well. The data was normalized against a no spermine condition, and the data was graphed **(Figure 8)**. Therefore, the data qualitatively and quantitatively differentiates between the two states.

	Spermine Concentration (ng/mL)			
	0.2 *Healthy	1.5	2 *Diseased	5
37° C				

Figure 7: Qualitative Spermine Data Differentiating Between Healthy and Diseased States. Image of assay when run with concentrations of spermine that exist at both the healthy and the diseased states.

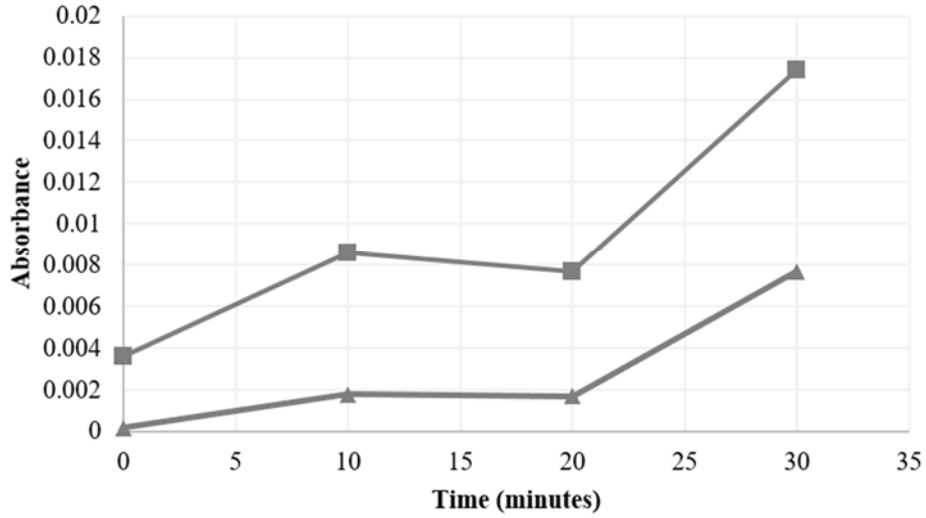


Figure 8: Quantitative Spermine Data Differentiating the Healthy and Diseased States. Healthy condition represented with triangles and diseased condition represented with squares.

4.3.2 Temperature Optimization

As done with the p16 ELISA assay, the spermine ELISA assay was also optimized for temperature. The ELISA assay required a 37 ℃ incubation. The assay was run at room temperature verify that it could be run without the use of any extraneous equipment that is not accessible in low-income settings such as in Tanzania. The tests indicated that the assay was still functional given that there

was a clearly more intense blue readout for the healthy individual compared to the lighter blue produced for the diseased individual at both temperatures. **(Figure 9)**.

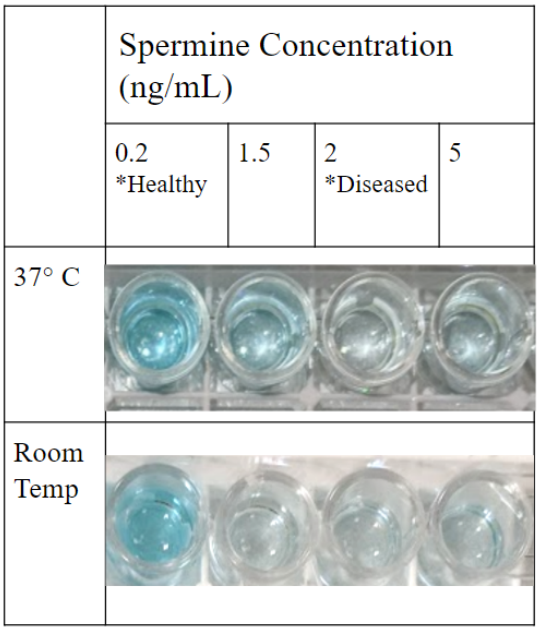


Figure 9: Qualitative Spermine Data for Temperature Optimization. Images of assay run at both the recommended temperature from distributor as well as at room temperature

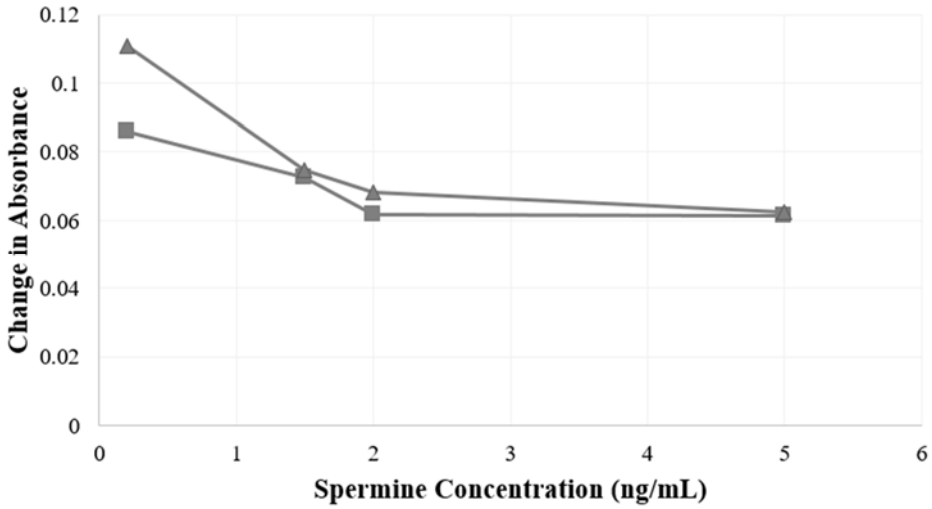


Figure 10: Quantitative Spermine Data for Temperature Optimization. 37 ℃ condition represented with triangles and room temperature condition represented with squares.

4.3.4 Fluid Optimization

As performed with the p16 ELISA assay above, the fluid used in the Spermine assay needed to be optimized to suit the non-invasive and urinalysis design of the biomarkers assays. We had to confirm that the results of the ELISA were comparably consistent for detecting the analyte in urine, as they were in the buffer that was provided. Therefore, to determine if the assay would be functional in detecting spermine in urine, we replaced the buffer with urine. Spermine was added to the urine at varying concentrations, and placed in the wells where the ELISA reaction took place **(Figure 11)**. Due to the competitive nature of the Spermine ELISA, presence of analyte was confirmed by an absence of color. Therefore, progression of color from dark to light confirmed that urine did not impede the reaction between the Spermine and the Spermine antibodies. Therefore, the Spermine ELISA assay is a functional assay for the detection of Spermine in urine.

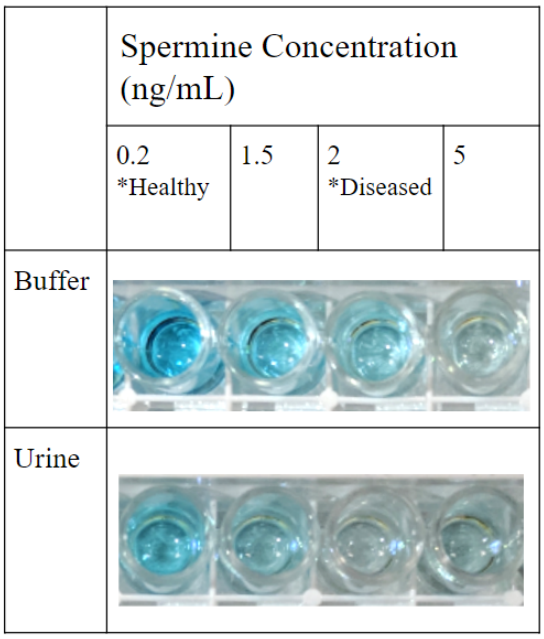


Figure 11: Qualitative Spermine Data for Fluid Optimization. Images of assays run in buffer supplied with ELISA kit and of assay run with artificial urine show no distinct difference.

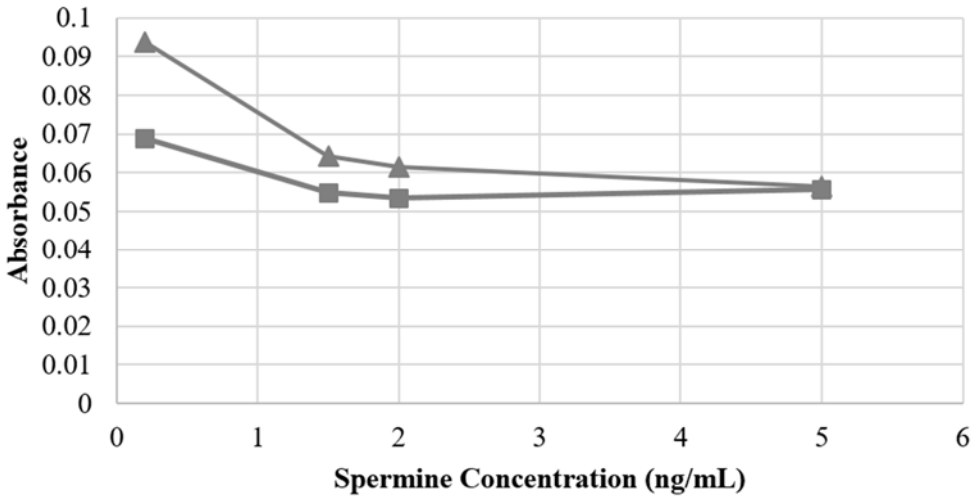


Figure 12: Quantitative Spermine Data for Fluid Optimization. Buffer condition represented with triangles and urine condition represented with squares.

4.4 Final Design Description

After testing the reagents that were necessary to screen for our biomarkers, we began researching how to incorporate these reagents into a lateral flow assay (LFA). A LFA uses similar reagents to ELISAs but the reagents are exposed to the fluid being analyzed through a different method. ELISAs are performed in a liquid solution with antibodies for the analyte being tested pre-coated to the bottom of the plate. These antibodies then bind to the analytes in the fluid being tested. Once the fluid being analyzed can interact with the pre-coated antibodies, a colored reagent specific to the analytes is added and subsequently binds to produce a colorimetric output that can be interpreted quantitatively and qualitatively.

LFAs require all the same biological reagents as an ELISA but instead utilize a solid technique that capitalizes on the flow of the fluid being analyzed through the assay itself. The fluid being analyzed reacts first with the colored reagents specific to the analytes being screened for. Then, as the fluid flows through the assay, they react with the antibodies that are specific to the analytes being screened for. These antibodies are printed in a single line and when they catch onto the analyte a visible color is produced due to the aggregation of the colored reagent attached to the

analyte. This method is solely qualitative. We are hoping to incorporate the antibodies from the ELISA into our LFA given that we have verified are capable of testing for our analytes.

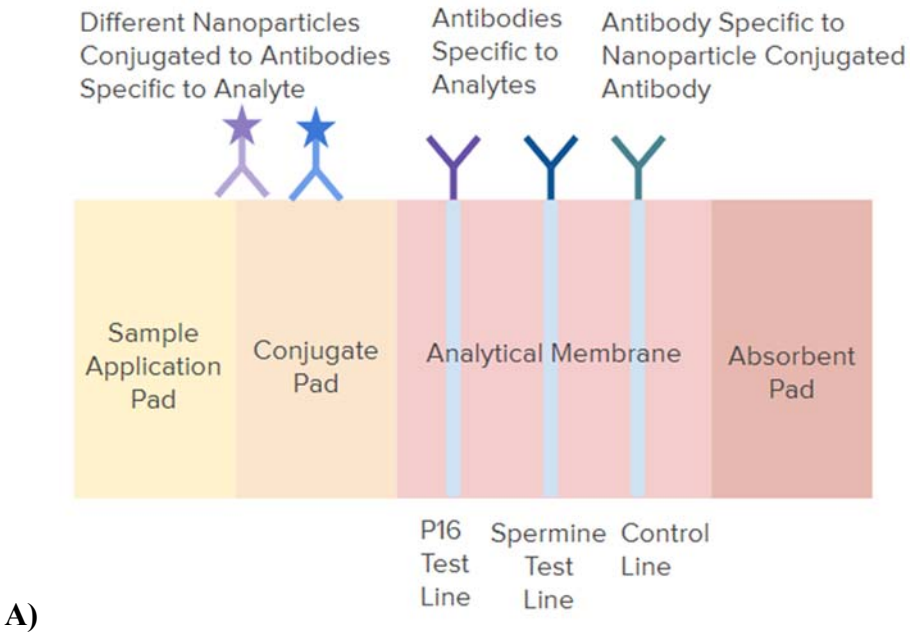
CHAPTER 5: System Integration

5.1 Lateral Flow Assay Components

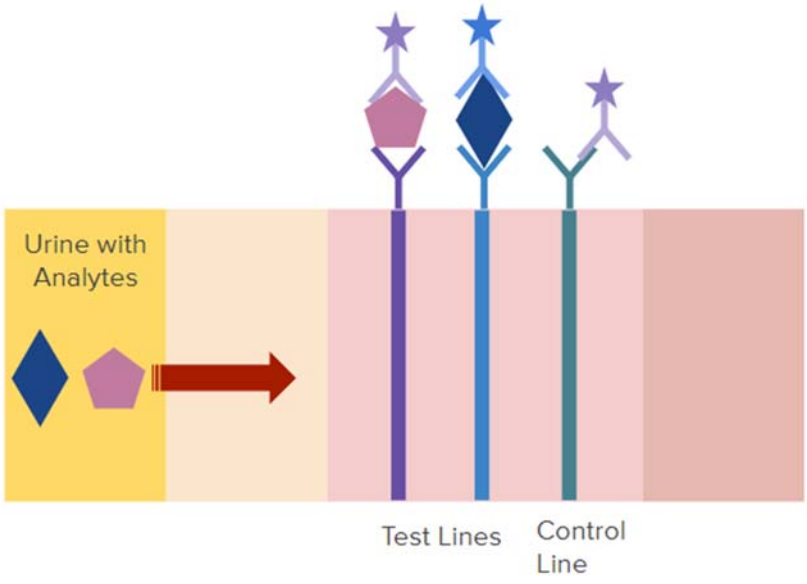
LFAs are beneficial for our purposes because these tests typically have long shelf lives and do not require refrigeration or special care, like many other diagnostic tests. Another benefit of an LFA is that it requires minimal training and no additional processing after use, which makes it well suited to act as a field based diagnostic necessary for our target population, Tanzania. Because these tests are powered only by capillary action, they do not require an energy source and provide a reading within 20 minutes of testing. Similarly, they only require a small amount of liquid sample to produce a result. In terms of sensitivity, LFA's have better sensitivity and specificity than other well-established methods under optimal conditions; however, depending on the analyte and antibody used there may be cases of low biomolecule affinity. [32]

A lateral flow assay works through multiple membranes with varying functions, which come together to allow for a qualitative colored readout that is easily understand by the user. The membranes and components in an LFA are pictured in **(Figure 13A)**. The first membrane is the sample application pad. The sample application pad is where the fluid to be analyzed is applied. In this case, our fluid would be urine, which the sample application pad is meant to filter as it moves through due to capillary action. The fluid then flows into the conjugate pad, which contains the colored reagents necessary to produce a colorimetric output. Often, colored nanoparticles are conjugated to antibodies specific to the biomolecules being screen for. These nanoparticles are then seeded onto the conjugate pad so that when the fluid runs through this pad, the antibodies will bind specifically to the molecules and move with them throughout the rest of the membrane. Another low-cost option that is sometimes used is Horseradish Peroxidase (HRP) which is the colored reagent we utilized in our ELISA assays. After the bind to the colored reagents, they then flow into the analytical membrane which contains test and control lines. Test lines contain antibodies that are specific to the biomolecules. When the analyte is present, it will bind to the control line, allowing for the colored nanoparticles to aggregate and produce a colored line that can be seen with naked eye. The control line contains antibodies that are specific to the conjugated nanoparticles. This final line is necessary to prove that even with no analyte present, the device is still functional as any fluid can move the particles seeded in the conjugate pad throughout the entire membrane to the test line. Our CERVIS LFA would have a

line specific to p16, a test line specific to spermine, and a control line specific to the antibodies conjugated to the colored particle of our choice **(Figure 13B)**.



A)



B)

Figure 13: Components of a Lateral Flow Assay. (A) Diagram of the different membranes and pads necessary to incorporate into a Lateral Flow Assay. **(B)** Diagram of LFA specific to CERVIS with urine being analyzed for the analytes p16 and spermine.

5.2 Optimization Process

There are various steps that must be taken to design a functional lateral flow assay. When designing a lateral flow assay, it is suggested to first design half stick assays before incorporating all pads and membranes necessary for a full LFA.

The half stick assay consists of the analytical membrane as well as the test and control lines. The analytical membrane is normally a nitrocellulose membrane. The properties of the nitrocellulose membrane used vary, so it is important to choose a membrane that works best for your test depending on the fluid you are analyzing, the analytes that are being screened for, as well as the functionality of the test. Membranes that are more porous will allow for the fluid to flow faster, producing a result faster. However, the speed may compromise the amount of time that the test lines can interact with the analytes present in solution, which could in turn impact how clear and intense the colorimetric result appears. Optimizing the half stick assay will not only require considering what membrane will be used but also which antibodies and colored reagents. We have already identified antibodies capable of screening for our analytes using ELISAs, however, throughout the LFA design process it may be useful to utilize antibodies from other manufacturers to observe which antibody produces the best result.

Once the half stick assay is optimized, the other components of the LFA can be incorporated and the entire assay can be further optimized. The sample pad will need to be optimized to filter out unwanted analytes. Materials often used are glass fiber, cellulose, cotton, and synthetic materials. The conjugate pad will be optimized by determining the volume of conjugate that can be absorbed onto the pad as well as released when fluid runs through it to ensure that there is no extra noise running through the analytical membrane that may produce unclear results.

CHAPTER 6: Costing Analysis

6.1 Cost of Materials for Development

As a device in the early stages of development, we still do not have an exact picture of the specific antibodies or materials that will be needed for the final device. However, a rough estimate can be made using the average costs of creating a lateral flow assay, and an estimation of the manufacturing price for our antibodies and antibody conjugated nanoparticles. Due to the existing mass production of lateral flow assays for a variety of purposes including pregnancy tests, glucose tests, biological threat agents, and HIV antibodies, there are companies that are already equipped to create and optimize each component of the lateral flow assay to the specifications of the analyte and antibody. As previously mentioned, the standard components of an LFA include the sample pad, the conjugate pad, the reaction matrix, the absorbent pad, and a membrane backing card. However, to manufacture an LFA for production and use also means the need for an upper casing, a membrane panel, a lower casing, a sample port, and protective housing for the device. Additionally, one will also need antibodies specific to the analyte, nanoparticle conjugated antibodies, and various reagents such as buffers, blocking agents, salts, etc. A report by *The Royal Society of Chemistry* estimates that in mass production, the sum of these components would average out to $0.22 per device. Almost 60% of this cost would be due to the cost of antibodies, 19% is attributed to the packaging that is needed for the pouch, 9% for the membrane backing cards, and the rest to the reagents, yarn costs, plastic housing, and manufacturing equipment and power. We expect our device to cost slightly more than this due to the use of multiple antibodies in parallel, however, our highest estimates keep the device under $1 per product, and most likely around the $0.50 mark. [32]

6.2 Expected Cost of Deployment

Similarly, to the cost of the materials, the cost of deployment of our diagnostic is still unclear to us as we proceed through the early stages of device creation and development. This will depend significantly on the final size of each diagnostic, the partnerships that we make with hospitals in Tanzania, and the strength of the connections that we form with nonprofits based in Tanzania who serve the more remote villages. An important factor to note is that although it may be relatively cheap to get the diagnostics from the United States to Tanzania, our partners at The Buturi Project, who personally service the Buturi region, a large region of Tanzania containing several villages,

have stated themselves that travel to and between each village is extremely difficult. There are few functional roads and vehicles for transportation, and the ones that do exist are neither cheap or reliable. Although it proved difficult to find existing examples of cost for lateral flow assays in Tanzania, we were able to locate a summary of transportation costs for the area. As according to a paper published by researchers of the Indian School of Business and University of California, Santa Cruz, the average village in Tanzania has 2,843 residents and is located 5.7 km from the nearest market center. This trip would cost approximately $1.60. However, an average village is located almost 65 km away from a major town, which increases the cost of the trip to $4.70. [33] Even though we consider that the cost would be divided among 20-100 devices, it would still add a significant amount to the cost of the device. Therefore, we would hope to manufacture the device within the country of Tanzania, and therefore eliminate any international transportation costs, and hopefully some of in the local costs as well.

CHAPTER 7: Engineering Standards and Ethics

The engineering standards that affect our design most are economic, manufacturability, social, and health and safety. Since we are planning our deploying our device in Tanzania, we had to take their economy into account. We sought to develop low cost device, so that people of all socioeconomic backgrounds would have the opportunity to get tested for cervical cancer.

Very early on in the process, we considered the manufacturability of our product. We want the product to be manufactured in the country of deployment, so that the clinics do not run out of the diagnostic while waiting for it to go through border control. Additionally, this would help the economy, as it would give the job of manufacturing the device to those who live in Tanzania.

The social considerations are a large factor in our project. Women's health is not well understood or discussed in Tanzania. Additionally, there is a stigma surrounding sexually transmitted diseases. This consideration inspired us to design an educational component that will be implemented with our product to educate those in the community about the risks of unprotected sex, the development from HPV to cervical cancer, and the necessity of getting tested regularly. Another social constraint we faced as invasiveness. We briefly considered making a device, similar to a tampon, that tested cervicovaginal fluid, but were concerned that it would not be socially acceptable.

The most important factor that we considered was health and safety. Our goal is to improve the health of women by enabling them to test for cervical cancer in its early stages. We want to help women around the world and facilitate the education of the relationship between cervical cancer, HPV, and sexually transmitted diseases. Since our product is a diagnostic, we are focused on making sure our test is sensitive and accurate, so it does not give false negatives and limited false positives.

Since our device is a cervical cancer diagnostic, the ethical concerns surrounding our device revolves around manufacturability and reliability. We must use Good Manufacturing Practices to develop and manufacture the product that will eventually be used to diagnose women with cervical cancer in various locations. Even though we are not treating women for cervical cancer, we must be certain that we have low rates of false positives and false negatives. False positives would result

if CERVIS says a woman has cervical cancer, when further tests confirm that she does not. False negatives occur when CERVIS says a woman does not have cervical cancer, when they do. Therefore, we must develop the device to limit the potential of false positives and false negatives and conduct iterative testing to prove accurate diagnoses. False negatives have a greater potential for harm than false positives, but it is important to limit both.

CHAPTER 8: Future Work

8.1 Global Health and Innovation Conference

In addition to conducting research and working in the lab to validate our assays and designing our LFA, we had the opportunity to pitch our product to a panel of experts, ask questions, and receive feedback at Unite for Sight's Global Health & Innovation Conference (GHIC). GHIC is the world's leading and largest global health conference as well as the largest social entrepreneurship conference that is held annually at Yale University. [34] We asked for advice on selecting biomarkers to differentiate HPV infection, CIN-1, and advanced cancer and for connections to advisors in our target population who can lend guidance on feasibility, field testing, and eventual adoption of our device. Although we were not able to receive advice on selecting additional biomarkers, we received feedback on expanding our target population to women who are victims of sexual abuse who may prefer a noninvasive option. Additionally, we were able to connect with CapraCare, a nonprofit organization in Haiti that educates people on cervical cancer, tests for the disease using visual inception by acetic acid (VIA), and treats it using cryotherapy. They are already gathering the data and have the infrastructure and connections we would need to deploy our device. We also made connections that will possibly result in additional funding. The conference contributed to our learning by expanded the scope of our project and reinforced the importance of this device. The product could save the lives of and positively impact a larger group of women than we previously thought possible.

8.2 Next Steps

Now that we have the reagents necessary to screen for our biomarkers, we are hoping to design an easy to use diagnostic known as a lateral flow assay (LFA). Currently, we have some of the reagents and materials necessary to design a lateral flow assay. We have multiple nitrocellulose membranes from Sartorius Membranes to optimize the analytical membrane. However, we must obtain antibodies specific to the analytes to create the test and control lines that will capture analytes flowing through the assay. Additionally, we must still purchase antibodies conjugated to colored nanoparticles that will produce the colored readout on the test and control lines. Once these reagents are obtained, half-stick assays can be developed to validate that the analytes can

still be measured using this new method. Finally, once the half-stick assays are validated, sample pads, conjugate pads, and absorbent pads must be obtained to develop the full lateral flow assay.

Additionally, we are in active communication with the professionals we met at the Global Health and Innovation Conference, through which we have the potential to establish a deployment strategy. We are hopeful that our device in combination with these connections will result in the prevention of unnecessary loss of life due to cervical cancer

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Appendices

Appendix A: Budget

Project Area	Material	Cost
Protein Detection	Protein detection kit (chemicals for detection of biomarkers)	$1000
	Filter paper	$50
	Standard bioengineering consumables (eppendorf tubes, pipette pins, well plates)	$50
	Simulated urine	$20
Existing products	Reagent Strips for Urinalysis 100ct	$50
Cost of actual protein	Mock P16 Inhibitor	$158
	CDKN2A Assay Kit	$548
	HRP	$76
	p16 ELISA	$435
	Spermine ELISA	$455
Amounts Requested	Requested from undergraduate grants	$1,800
	Requested from Xilinx grant	$1000

	Total Requested	$2,800
Amounts Received	Grants awarded by School of Engineering	$1,500
	Grants awarded by Engineering World Health	$1,000
	Total Awarded	$2,500

Appendix B: Gannt Chart

PROJECT TITLE	Cervical Cancer Early Response Visual Identificatic
PROJECT MANAGER	Dr. Asuri

TASK TITLE	PCT OF TASK COMPLETE	Fall Quarter											Winter Quarter											Spring Quarter										
		1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11
Project Definition and Planning																																		
Initial project selection	100%																																	
Determine acceptance criteria	100%																																	
Background cervical cancer presentation	100%																																	
Final project selection	100%																																	
Fall Quarter Presentation	100%																																	
Introduction chapter completion	100%																																	
Project Launch & Execution																																		
Researching biomarkers	100%																																	
Successful assay identification	100%																																	
Spermine ELISA	100%																																	
p16 ELISA	100%																																	
Assay optimization	100%																																	
Project Performance/Monitoring																																		
Progress Presentation	100%																																	
GHIC conference	100%																																	
LFA testing	70%																																	
LFA optimization	10%																																	
Senior Design conference	100%																																	
Senior thesis completion	90%																																	

Appendix C: Proposed p16 ADP GLO Protocol

Kinase Reaction for Cdk2/Cyclin A2 kinase enzyme system

Normally in a 96 well plate they use 25 µL as the total reaction volume

Make a 100 µL kinase reaction and then split into 4 well plates for a 25 µL: 25 µL: 50 µL final reaction to record luminescence

- Initial prep
    - Dilute 0.5 µL of 0.1M DTT in 200 µL of 5x reaction buffer to form Reaction Buffer A
- Kinase reaction
    - Control reaction: 100 µL to be split into 4 trials
        - Add 75.5 µL of DI water to 20 µL Reaction Buffer A
        - Add 4 µL Histone H1 to reaction
        - Add 0.5 µL of ultra pure ATP
        - 0 µL of Kinase
    - 0.63 Kinase Reaction: 100 µL to be split into 4 trials
        - Add 74.9 µL of DI water to 20 µL Reaction Buffer A
        - Add 4 µL Histone H1 to reaction
        - Add 0.5 µL of ultra-pure ATP
        - Add 0.63 µL of Kinase
    - 1.26 Kinase Reaction: 100 µL to be split into 4 trials
        - Add 74.2 µL of DI water to 20 µL Reaction Buffer A
        - Add 4 µL Histone H1 to reaction
        - Add 0.5 µL of ultra-pure ATP
        - Add 1.26 µL of Kinase
    - 3 Kinase Reaction: 100 µL to be split into 4 trials
        - Add 72.5 µL of DI water to 20 µL of Reaction Buffer A
        - Add 4 µL Histone H1 to reaction
        - Add 0.5 µL of ultra-pure ATP
        - Add 3 µL of Kinase

    - Incubate for 10 minutes
- ADP depletion
    - Add 100 µL of ADP Glo Reagent (1:1:2 of Kinase Rxn:ADP Glo: Kinase Detection Reagent)
    - Incubate for 40 minutes
- ADP detection
    - Add 50 µL of reaction to 4 wells
    - Add 50 µL of Kinase Detection Reagent to each of the 4 wells
    - Incubate for 30 minutes
- Spectrophotometer readings
    - Take absorbance readings of well plate
    - Added Kinase Detection Reagent into two separate wells (50 µL) to act as a blank
    - Take absorbance readings again
    - Take luminescence readings

Appendix D: ELISA Protocols

Appendix D1: P16 ELISA

Sensitivity of this assay is 6.25 pg/mL - 200 pg/mL.

1. Bring all reagents and samples to room temperature (18 °C-25 °C) naturally for 30 min before starting assay procedures. The MicroELISA Strip plate is detachable, detach unused strips from the plate frame, return them to the foil pouch with the desiccant pack, and reseal for preventing damps.
2. Standard wells, Sample wells and Blank/Control wells
    a. Add Standard 50 µL to each Standard well
    b. Add Sample 50 µL to each sample well
    c. Add Sample Diluent 50 µL to each Blank/Control well.
3. Add 100 µL of HRP-conjugate reagent to each well, cover with a Closure Plate Membrane and incubate for 60 minutes at 37 °C or room temperature.
4. Wash the plate 4 times in wash buffer (dilute 20x to 1x using deionized water):
    a. Manual Washing – Dump the incubation mixtures of the wells into a sink or proper waste container. Using pipette or squirt bottle, fill each well completely with Wash Solution (1x), after about one minute's standing, invert and hit the plate onto absorbent papers or paper towels until no moisture appears. Repeat this procedure four times.
5. Add Chromogen Solution A 50 µL and Chromogen Solution B 50 µL to each well successively and protect from light to incubate for 15 minutes at 37 °C or room temperature.
6. Read the optical density at 450 nm using plate reader**.**

Appendix D2: Spermine ELISA

Sensitivity of this assay is 0.5 - 10 ng/mL

1. Bring all reagents and samples to room temperature (18 °C-25 °C) naturally for 30 min before starting assay procedures.
2. Standard wells, Sample wells and Blank/Control wells
    a. add Standard 100 µL to each Standard well
    b. add Sample 100 µL to each sample well and 10 µL of balance solution

c. add 100 μL of PBS into the blank control well

3. Add 50 μL of conjugate to each well and mix thoroughly. Cover and incubate the plate for 1 hour at 37 °C or at room temperature.
4. Wash the plate 4 times in wash buffer (dilute 20x to 1x using deionized water):
    a. Manual Washing – Dump the incubation mixtures of the wells into a sink or proper waste container. Using pipette or squirt bottle, fill each well completely with Wash Solution (1x), after about one minute's standing, invert and hit the plate onto absorbent papers or paper towels until no moisture appears. Repeat this procedure four times.
5. Add Substrate A 50 μL and Substrate B 50 μL to each well successively and protect from light to incubate for 15 minutes at 37 °C or at room temperature.
6. Read the optical density at 450 nm using a plate reader.

Appendix E: Assessment of Current Diagnostic Options

The rating system is from 0, being the lowest, to 5, being the highest. We believe the factors each diagnostic is ranked against are important in a successful and high-impact product. The rankings were determined after extensive review of the methods of these technologies and outcomes. If the current diagnostic completely misses one of our requirements, they received a score of 0 or 1 in that factor. Additionally, the ranking generally compares one diagnostic method against the others and considers ease of use.

Table 1: Evaluation of Current Technologies Utilized for Diagnosing Cervical Cancer

	Visual Inspection by Acetic Acid / Iodine	**Pap Test**	**Colposcopy**	**Roche CINtec Histology Test**
Minimal Invasiveness	**3** Requires only one visit.	**1** Metal / plastic speculum used to open walls of vagina to get to cervix. Sampler collects cells to be tested. Multiple visits necessary.	**1** Closer examination of cervix. Colposcope used. Only done when abnormal pap test result obtained.	**2** Tissue biopsy necessary. The assay is only run if there are abnormalities.
High Success Rate	**2** Resources used on unnecessary treatment of women who are free of	**2** Only frequent cervical screening can detect HPV infections.	**3** More specific analysis of histology and cytology following a pap	**5** FDA approved with the ATHENA trial that surveyed more than

	precancerous lesions in a single-visit approach.		test.	47,000 women *Specific to HPV
Easy Accessibility	**5** Minimal infrastructure needed: exam area and table, trained health professionals, speculum, and acetic acid.	**2** Not easily accessible in low-income countries. Follow up tests necessary with doctors 1 yr. and 3-5 yrs.	**2** Not easily accessible in low-income countries.	**2** Not easily accessible in low-income countries.
Low Cost	**5** Low start-up and sustaining costs.	**3** $50 to $200 per test.	**1** $100-800 per test (already following pap test).	**-** Not enough information on cost to be assessed.
High Accuracy	**2** Naked eye used to identify color changes on cervix after application of chemical	**3** Looks solely at histology of the cells. Follow up tests are necessary. Needs to be in conjunction with an HPV	**3** Looks solely at histology of the cells.	**4** Numerous biomarkers used, including p16, which is recommended by the World Health Organization.

		test.		
Expertise Necessary	**3** Trained health professionals required to perform the procedure.	**1** Specialized physician required to carry out procedures.	**1** Specialized physician required to carry out procedures.	**2** Specialized physician required to interpret results.
Focus on Cervical Cancer over HPV	**2** Not specific to cervical cancer and detects for HPV.	**2** Need to run Pap test in conjunction with HPV test for best results.	**3** This examination done after having abnormal pap smear. Positive results in the need for a Cervical biopsy.	**2** Used to confirm the presence or absence of high-grade cervical lesions utilizing HPV specific markers.