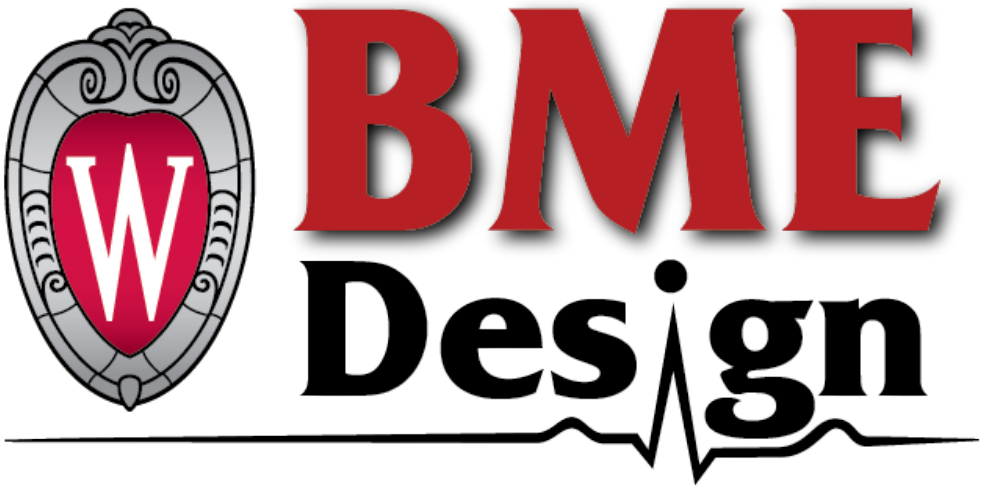

Non-Invasive Cervical Cancer Screening

Final Report

BME 200/300

Team Members:
Georgia Hancock, Team Leader
Mira Baichoo, Communicator
Karina Buttram, BPAG
Josephine Hall, BSAC
Adrienne Simpson, Co-BWIG
Cora Williams, Co-BWIG

Client:
Kebron Zegeye

Advisor:
Dr. Tongcheng Qian

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Table of Contents

I. Abstract

Cervical cancer is one of the most prevalent types of cancer in women, and also the most treatable when detected early. Current methods of screening for early detection are limited, and those available are uncomfortable and inaccessible to many women including those in rural Ethiopia. Many women in this region lack access to healthcare facilities where typical screenings are performed, meaning many cases of developing cervical cancer go undiagnosed.

The team developed a device that can test for cervical cancer markers at-home, using a self-collected urine sample to test for the presence of oncoproteins E6 and E7. Presence of these oncoproteins in urine is highly indicative of cervical cancer and a positive test result should prompt the user to seek further treatment. Positive results are indicated by a color change on the test using a lateral flow assay.

It is necessary to ensure that women in rural Ethiopia and globally have access to the necessary healthcare to maintain their reproductive and overall health regardless of geographic location or socioeconomic status. This device will allow women to have more autonomy surrounding their health by providing a more comfortable and more convenient alternative to invasive testing methods at a low cost.

II. Introduction

Motivation

Cervical cancer is one of the most prevalent cancers in women while also being one of the most treatable cancers when diagnosed early [1]. This type of cancer affects the lowermost part of the uterus, also known as the cervix, and is caused by long-term human papillomavirus (HPV) infection. HPV accounts for 91% of all cervical cancer [2]. There are many strains of HPV; however, HPV 16 and 18 are the two most predominant cancer-causing strains [3]. The top 20 countries with the highest rates of cervical cancer are within Africa [4]. The causes of this can be linked to limited access to hospitals and clinics, limited funds to pay for testing, and the cultural taboo around women's health [5].

Current Methods

Current testing methods for cervical cancer include routine Pap smears, and less commonly, oncoprotein testing. A Pap smear is a procedure where a provider inserts a speculum to visualize the cervix and uses a wooden or plastic scraper and/or cervical brush to collect cell samples [6]. The cell sample is then placed under a microscope where a medical professional screens for abnormal cells. Another less common screening method is the OncoE6 cervical cancer test, which is a rapid cervical cell sample test based on the detection of E6 oncoproteins. This qualitative test is used to analyze cells extracted from cervical cytology swab specimens obtained by a medical professional. It uses highly specific monoclonal antibodies (mAbs) in a

lateral-flow (LF) assay format to detect these oncoproteins. It is available in the US but must be completed through the manufacturer's CLIA-certified laboratory [7].

Problem Statement

Although current testing methods are somewhat successful at detecting cervical cancer, they are uncomfortable and not easily accessible or affordable for people in developing countries. The aim of this project is to create a non-invasive cervical cancer screening device utilizing a self-collected sample that could be performed and analyzed at home without the use of laboratory techniques. This testing device will be available to women in rural Ethiopia as an inexpensive and accessible method of regular screening for early cervical cancer markers, allowing more women in these areas to be informed about their health and take steps towards treatment if necessary. It would reduce the rate of cervical cancer cases that go undiagnosed and therefore improve long term patient outcomes.

III. Background

Background Research

Human Papillomavirus (HPV) is the most common sexually transmitted infection. There are 200 different strains of HPV, but only 40 can infect the genital area [8]. Persisting infection of certain strains of HPV can lead to cervical cancer in women. The two strains of most concern when considering the potential of cervical cancer are HPV 16 and HPV 18. HPV 16 is linked to approximately 50% of cervical cancer cases worldwide and HPV 18 is the second most prominent strain [8].

After extensive research, it was determined that the oncoproteins E6 and E7 would be prime candidates for testing. E6 and E7 are two oncoproteins that promote cell proliferation and survival [9]. Both oncoproteins target tumor suppressor proteins and effectively inactivate them, allowing for malignant cell proliferation [10]. Establishment and progression of HPV caused cervical cancer requires the presence of E6 and E7 oncoproteins [11]. The E6 and E7 encoding DNA in HPV is highly conserved across all strains, meaning E6 and E7 will have limited to no mutations [12]. A benefit of screening for E6 and E7 is their early detection, which can be seen 20-30 years before the HPV infection progresses into cervical cancer [13].

Both E6 and E7 bind to specific proteins found in the human body (p53 and pRB respectively) [14] [15]. E6 must first bind to E6AP, which in turn opens a binding site on E6 where it can attach to p53 [14]. E7 does not follow the same binding pattern as E6 in that E7 can bind to pRB and, theoretically, bind to the anti-E7 antibody simultaneously [15]. The binding patterns exhibited by E6 and E7 were utilized in the device creation.

Pregnancy tests are common at-home urine sample testing devices that use a lateral flow sandwich assay. The test uses Rabbit IgG antibodies coated in blue latex particles to create the blue control line, which is visible on the test strip when bound to Goat-anti Rabbit IgG

antibodies. The control line ensures that the test is functioning properly and a valid urine sample has been deposited into the testing device [16]. The creation of a control line on the cervical cancer detection device was modeled off of the pregnancy test control line.

Client Information

The client, Ms. Kebron Zeygeye, is a Biomedical Engineer based in Ethiopia. She was motivated to start this project because of her personal connection to women affected by cervical cancer that went undiagnosed.

Product Design Specifications

This device must be a non-invasive cervical cancer test that can be completed by the user in an at-home setting. It must not require the sample to be collected by a medical professional and can not utilize laboratory techniques to determine results. All components of the test that may come in contact with the user must be biocompatible and all materials should be non-biodegradable. The purchase cost to the consumer should be between $3-$5 USD. More detailed requirements and specifications are included in the Product Design Specifications in Appendix A.

IV. Preliminary Designs

Strip Dip

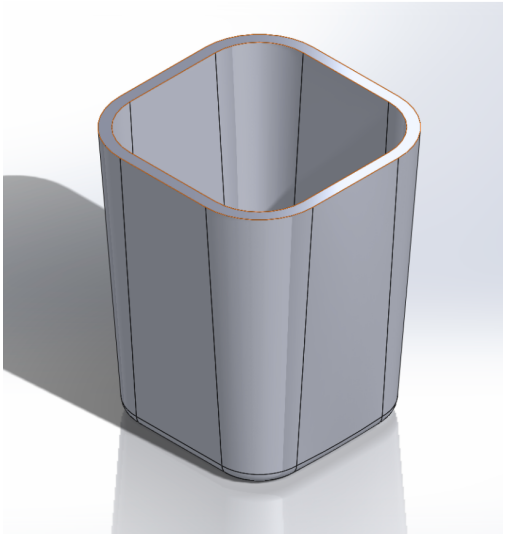

Figure 1. Strip Dip Collection Device

The first preliminary design is the Strip Dip collection device. It contains a cup that will hold the user's urine and a test strip with an absorbent pad. The test strip will be submerged into

the sample and laid flat to allow the sample to move through the strip. The strip will produce a color change indicating a positive result if the sample contains biomarkers indicative of cervical cancer.

Drop Test

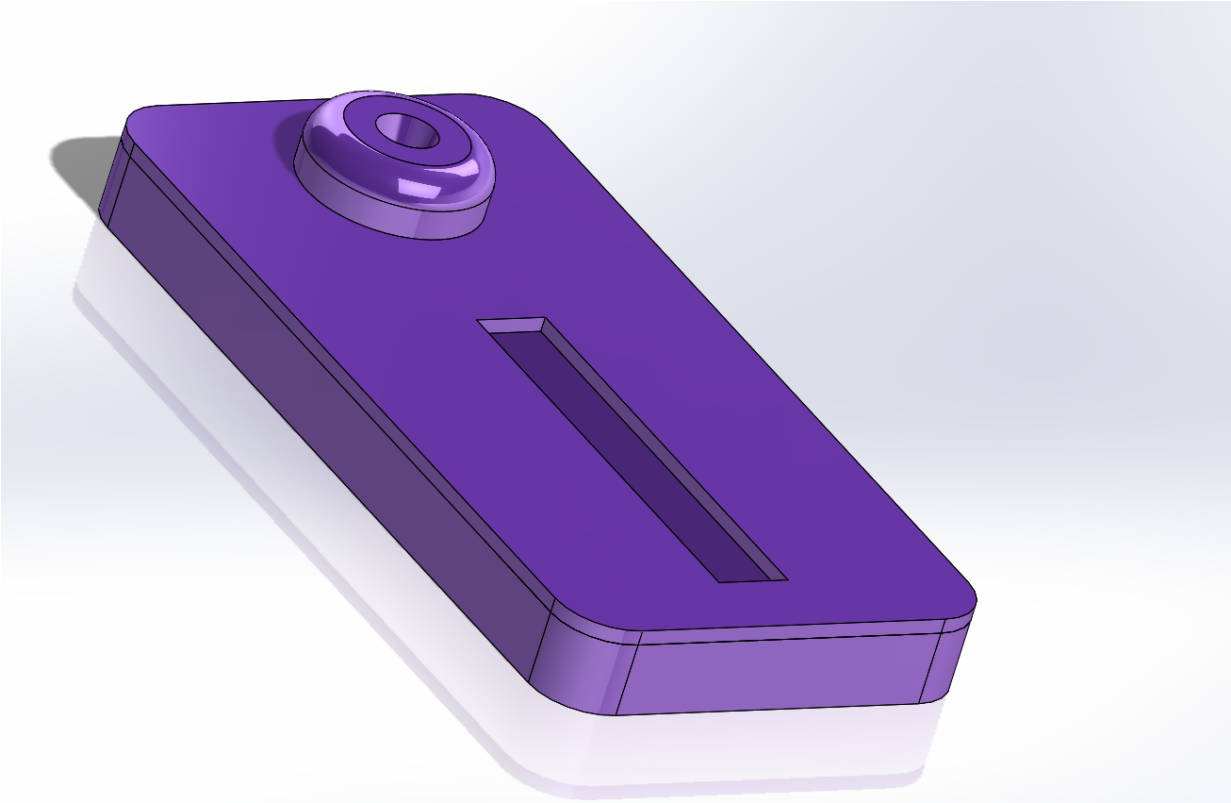

Figure 2. Drop Test Collection Device

The second preliminary design is the Drop Test collection device. The user will collect the sample in a provided cup and use a simple pipette to transfer the sample to the device by inserting the tip into the hole. This will funnel the sample directly onto the absorbent pad. The sample will then flow down the test strip, producing a result via a color change that can be viewed through the rectangular hole in the top of the device.

Funnel Device

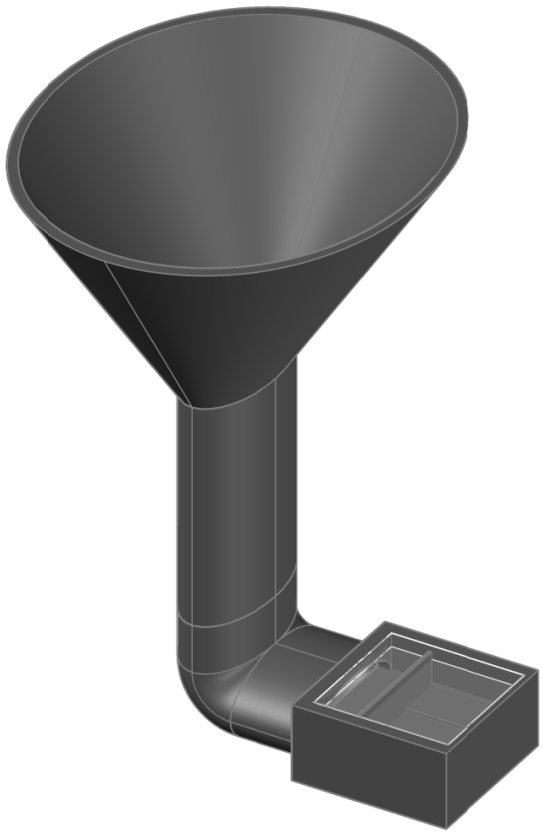

Figure 3. Funnel Device

The third preliminary design is the Funnel Device collection method. This design is similar to Drop Test design in that it contains a test strip coated with a reagent encased in a plastic housing. The plastic container with the strip is connected to a funnel where the user urinates into. The sample moves down the funnel to reach the absorbent pad and then flows through the strip. A color change for a positive result will be visible through the clear plastic top of the test strip housing. It contains a divider between the absorbent pad and the reagent-coated strip that prevents urine from flooding the reagent side and only allows it to pass through the absorbent pad.

V. Preliminary Design Evaluation

Table 1: Sample Type Design Matrix

Designs **Categories**	**#1 Blood**		**#2 Saliva**		**#3 Urine**	
Prior Detection (30)	3/5	18	3/5	18	4/5	24
Ease of Obtaining Usable Sample (25)	4/5	20	3/5	15	4/5	20
Comfort (20)	2/5	8	5/5	20	4/5	16
Ease of Collection (15)	2/5	6	5/5	15	4/5	12
Storage Requirements (10)	2/5	4	5/5	10	4/5	8
Total (100)	56		78		**80**	

The first design matrix compares different sample types based on criteria that would make them most compatible with the project goals. The highest ranked criteria was prior detection to ensure a sample was chosen that had adequate previous experimentation indicating the testable presence of HPV. Urine ranked the highest of the samples as the team's research proved it was reliable in containing HPV markers. The next highest criteria is the ease of obtaining a usable sample, where saliva ranked lower than blood and urine due to a higher possibility of collecting contaminants. The next highest criteria was comfort as the main goal of the product is to provide a more comfortable alternative to a Pap smear. Saliva ranked highest as spitting would be the most comfortable and noninvasive method. The final two criteria are ease of collection and storage requirements. Of all the samples, urine scored the highest against the given criteria and was the chosen sample type. Urine scored the highest in our design matrix as it received the highest scores in prior detection and ease of obtaining a usable sample. Urine also scored well in the three remaining categories and was deemed to be a more reliable option compared to the second choice of saliva, as saliva has more potential for food contamination.

Table 2: Testing Apparatus Design Matrix

Designs / **Categories**	**#1 Strip Dip**		**#2 Drop Test**		**#3 Funnel Device**	
Ease of Use (30)	3/5	18	4/5	24	**5/5**	**30**
Cost (25)	**5/5**	**25**	3/5	15	2/5	10
Ease of Fabrication (20)	**5/5**	**20**	4/5	16	2/5	8
Sample Containment (15)	3/5	9	**5/5**	**15**	2/5	6
Efficiency (10)	4/5	10	**5/5**	**10**	3/5	6
Total (100)	**82**		**80**		60	

For the collection device design matrix, the highest ranked criteria was ease of us. The funnel device ranked the highest because the user needs to urinate into a funnel which immediately puts urine in contact with the test, making this a single step process. The next highest ranked criteria was cost. The main target demographic for this product is from rural or low-income areas where cost can become a barrier. The product cost has to be low enough to allow easy access for users of varying socioeconomic backgrounds. Strip dip was highest in this category because it would be the most economical to price only a cup and test stick between 3 and 5 dollars, since it does not require a custom device to house the test strip. The next category was ease of fabrication to ensure feasibility of creating a usable prototype. The highest was the strip dip because the only components needed were a cup and the test strip, neither of which require any complicated designs or fabrication. The next category was sample containment because the test needs to be able to maintain the correct volume of the sample for the duration of testing. The drop test was the highest of this category because the casing and dripping onto the pad would allow the sample to remain at the same volume until the test is done with little chance of oversaturation. The last category was efficiency as the design should be the easiest to construct and get to consumers. The highest ranked was the drop test because it is rectangular in shape, allowing for easy packaging and shipping, as well as being a discrete design. Overall, the strip dip ranked the highest based on the designated criteria; however, the drop test was chosen

as the final design because it allows for a more secure testing method with a protected strip and specified sample volume. See **Figures 1,2, and 3** for images of the proposed collection devices.

VI. Fabrication/Development Process

Materials for Physical Testing

The materials for the physical testing apparatus includes 1 clear cup, 1 3 mL pipette, and 1 testing device casing. The clear, plastic cup is intended for the user to fill with their urine sample. The user will then use the 3 mL, single use pipette to transfer 0.5 mL of urine into the insertion bubble of the collection mechanism. The testing device casing will be 3D printed from a "TOUGH" resin filament and used to house the nitrocellulose test strip with an attached absorbent pad for the lateral flow assay.

Materials for Biomarker Testing

A nitrocellulose strip with an attached absorbent pad will be used to indicate a positive or negative result for the presence of E6 and E7, via a color change. Placed at the top of the strip will be Rabbit IgG, E6AP, and pRB antibodies coated in blue latex particles. Further down the test strip, the Goat-anti Rabbit IgG, p53 antibodies, and anti-E7 antibodies will be absorbed into the test strip by applying them in a solution. After the antibody solutions have been absorbed, they will be adhered to the strip in parallel lines, with one line indicating the control line, one line indicating a positive E6 result, and one line indicating a positive E7 result.

Methods

With a prototype designed in SolidWorks, a 3D print of the collection mechanism will be made using "TOUGH" resin filament. The casing will be printed in two pieces (base and lid), to ensure that the device is hollow. The cup for collecting urine and the pipette for the transfer of urine do not require any fabrication. After the device casing is printed, the test strip will be developed.

The nitrocellulose test strip will contain an attached absorbent pad on the end. Closest to the absorbent pad, the Rabbit IgG, E6AP antibodies, and pRb antibodies will be coated in blue latex particles and placed on the test strip. Each of these three antibodies will be placed in a vial with the blue latex particles in order to thoroughly coat each antibody before being placed on the test strip. On the other end of the test strip, the Goat-anti Rabbit IgG, p53 antibodies, and anti-E7 antibodies will first be placed in a solution and then applied to the test strip in parallel lines. The antibody solutions will absorb into the test strip to ensure these three antibodies will be immobile on the strip. The Goat-anti Rabbit IgG blue line will indicate the control line to ensure the user that the test is working properly and a valid urine sample has been deposited into the device. The p53 antibody blue line will indicate a positive E6 result, and the anti-E7 antibody blue line will indicate a positive E7 result.

Once the test strip contains all six of the antibodies and the testing device casing is printed, the nitrocellulose test strip will be placed inside the base of the casing. The absorbent pad of the test strip will be on the same end as the insertion bubble on the lid of the casing. Once the test strip is in place, the lid of the casing will be superglued to the base and the biomarker testing device will be fully assembled.

Final Prototype

The final prototype fabricated by the team was the drop test device with an interior nitrocellulose test strip which utilizes a lateral flow sandwich assay to produce a color change result for both E6 and E7 oncoproteins, along with a control line. The complete test kit includes a small cup for the user to deposit the sample, as well as a simple 3mL pipette dropper to deposit their sample onto the absorbent pad with increased precision using the funnel hole.

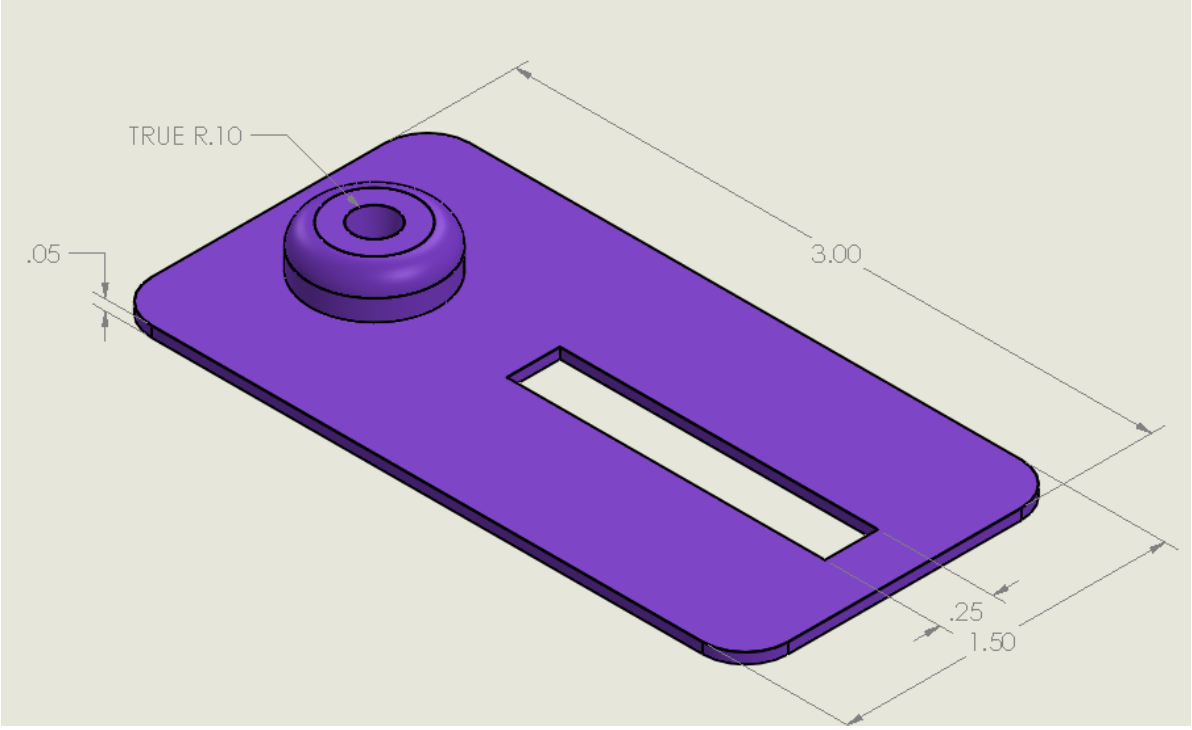

Figure 4. Final Prototype Lid

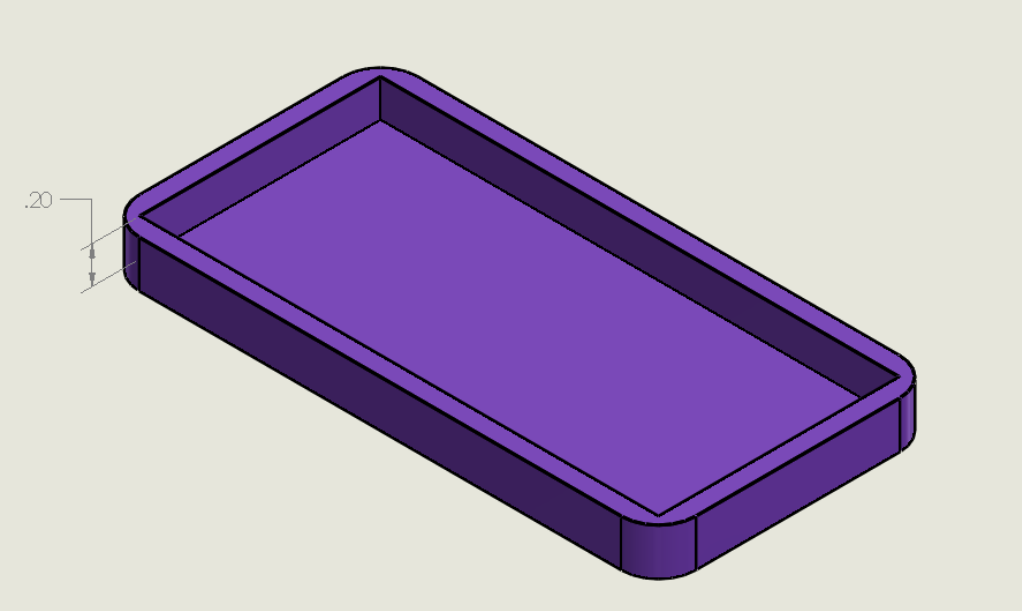

Figure 5. Final Prototype Base

The test housing was modified from the initial prototype by expanding the size of the sample insertion hole as well as upgrading material from PLA to a smoother and more rigid resin material, “TOUGH”. It was sized appropriately to fit in the user’s hand with depth minimized to allow easier packaging while still having enough thickness to hold the necessary amount of sample and remain easy to handle during use. This housing was 3D printed in two pieces to allow the final prototype to be hollow. The print was separated into a base piece where the nitrocellulose strip lies as well as a “lid” including the bubble in which the dropper is inserted for sample application as well as the viewing hole. The device was assembled by using gorilla glue to attach the lid piece to the base.

Within the housing is a nitrocellulose test strip and an absorbent pad below the insertion hole. After analyzing previously published studies, the team decided to test for the presence of both E6 and E7 oncoproteins, as the test accuracy of previous tests looking for only E6 was too low for the project specifications set out by the client [17]. At the top of the strip are colored latex-coated antibodies E6AP and pRb which bind to oncoproteins E6 and E7 along with rabbit IgG antibodies. These move down the strip with the urine towards 3 seperate sites with bound antibodies p53, anti-E7, and goat-anti rabbit IgG which bind to E6, E7, and rabbit IgG, respectively. The rabbit IgG will bind to goat-anti rabbit IgG to create the first blue line on the test regardless of the content of the sample. This is for the purpose of ensuring the test is still functional. If E6 is present, E6 will bind to both E6AP and p53 to produce a second blue line behind the control line on the nitrocellulose strip. If E7 is present, E7 will bind to both pRB and anti-E7 creating a blue line behind both the control line and the p53 antibody adherence location. If both oncoproteins are present, the test will present 3 blue lines.

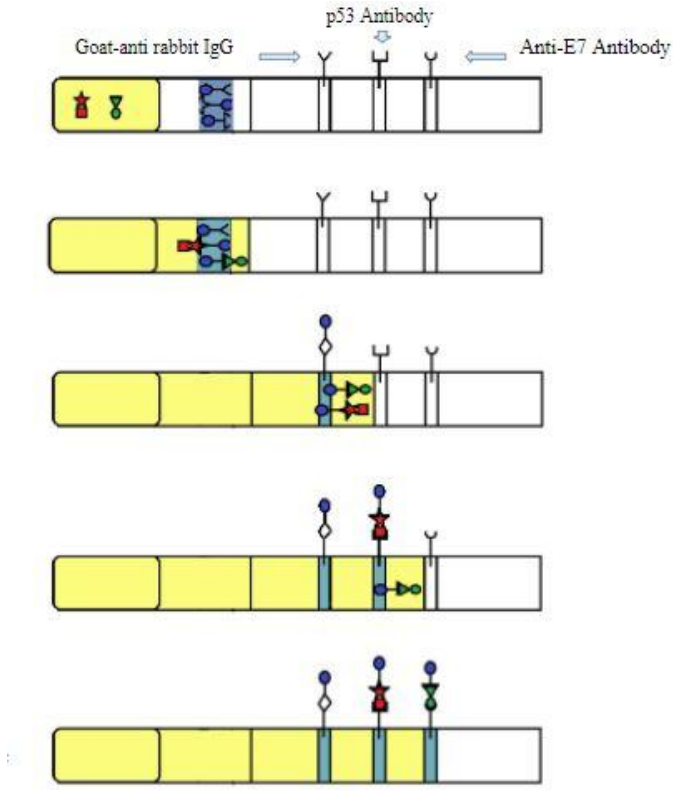

Figure 6. Schematic of function of Lateral flow assay.

If both E6 and E7 are present, the red body (E6) and the green body (E7) move down the test strip with the movement of the urine. They bind to latex bound pRB and E6AP antibodies. The control line appears due to the binding of latex coated Rabbit IgG and Goat-anti Rabbit IgG antibodies. E6 finally binds to p53, creating a blue line where the p53 antibody is adhered. E7 then binds to the anti-E7 antibody creating an additional blue line.

Testing

To assess potential mechanical failures and prototype durability, the test device casing must be handled with slight pressure to simulate typical use. All components should stay in place and the housing should not permanently deform in any way.

Device leakage must be tested to ensure security of the sample. No liquid should escape the housing after the user deposits the sample. Water should be dropped into the device casing using a pipette to test for leakage in place of a urine sample. A 0.5 mL water sample should be tested to simulate the recommended sample size from the user. A 3 mL water sample should also be tested to simulate user error. There should be no leakage observed after two minutes.

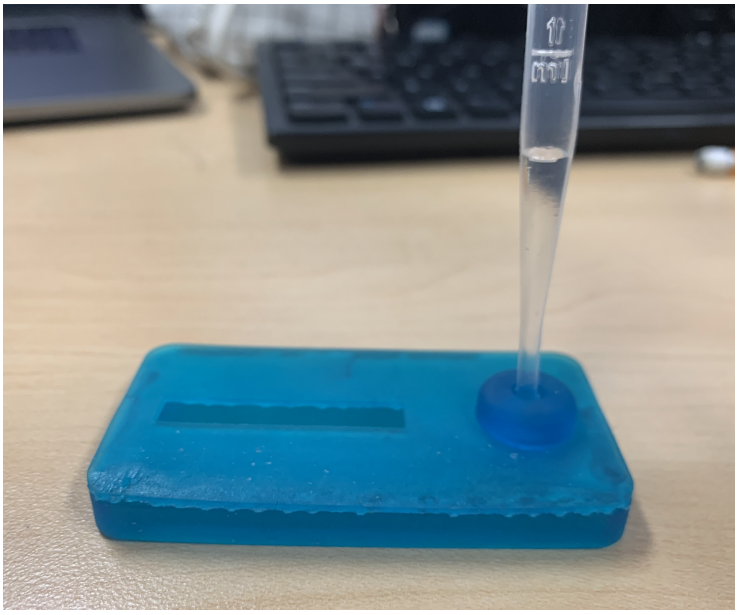

Figure 7. Leakage Test in Progress

To test for the biological samples and the accuracy, multiple more extensive tests that are beyond the current resources of this project would need to be performed. These tests include absorbent pad testing, concentration of biomarkers in urine tests, line visibility testing, multiple HPV strain tests, and accuracy testing to determine if both E6 and E7 are necessary for the device and if the result accuracy meets the product requirements. More information on these future tests and generalized protocols can be found in the appendix D.

VII. Results

The original prototype made of PLA filament failed the durability test, since the smaller insertion bubble did not print correctly and the bubble collapsed upon handling of the device. The prototype was updated so that the insertion bubble is bigger and printed from a more rigid material, "TOUGH" resin. Upon testing of the updated prototype, the bubble held up against hand pressure and the "TOUGH" resin prototype resulted in a higher quality print than the original prototype.

The leakage test was conducted on our resin prototype using two different volumes of water representing correct and incorrect use. After two minutes of observation, neither the 0.5 mL nor the 3 mL sample leaked out of the device, confirming that this prototype passed the leakage test.

The results of the biomarker strip tests will determine the accuracy of the test, which should be greater than 70%. More information on these results and potential modifications based on the results can be found in appendix E.

VIII. Discussion

This research has led the team to focus on HPV E6 and E7 oncoproteins as biomarkers for HPV and cervical cancer in urine. Urine has not previously been used to detect cervical cancer in a home environment, because many testing methods often require the use of laboratory techniques such as methylation and DNA sequencing [18]. The OncoE6 Cervical Cancer Test uses laboratory techniques and tests for HPV E6 oncoprotein in cervical cell samples [19]. This testing method detects less than 50% of positive cervical cancer cases [20]. The low accuracy rate of the OncoE6 Cervical Cancer Test will be used as a benchmark for further accuracy testing of the at-home, self-collected urine sample device.

Due to the inability for undergraduate students without proper training to handle human specimens, testing the device with HPV positive urine samples was not feasible. Additionally, without any laboratory access or faculty connections with UW Hospitals, it is extremely difficult to obtain any HPV positive urine samples to test the accuracy of the at-home, self-collected urine sample device.

Future testing would require individuals willing to disclose their health records and be willing to participate in medical research studies. This creates an ethical constraint as individuals have a right to medical privacy.

IX. Conclusions

Summary

The team was tasked with creating a cervical cancer testing device that can be completed at home and does not utilize laboratory techniques. The final test kit prototype consisted of a 3-D printed testing device casing, biomarker nitrocellulose strip, sterile sample-collection cup, and a sterile pipette. The user would deposit a urine sample into the sample-collection cup, using the pipette to transfer the urine sample from the cup by inserting it into the extruded hole on the testing device casing. Once the urine is deposited into the device, the urine will be absorbed by the absorbent pad connected to the nitrocellulose strip. The urine is then wicked down the nitrocellulose strip where it participates in a sandwich lateral flow immunochromatographic assay test, determining if E6 and E7 oncoproteins are present via a color change in different locations on the strip corresponding to each biomarker.

The team decided to screen for cervical cancer using E6 and E7 as biomarkers. E6 and E7 are found in all cervical cancer cases, as they are oncoproteins associated in HPV. This decision was also based upon the uses of E6 and E7 in many other research studies testing for cervical cancer, which proved that these biomarkers would be a viable option.

Due to durability requirements, the team decided to print the testing device casing out of “TOUGH” resin material. This decision was made after the initial prototype, which was printed out of PLA filament, broke easily in the durability test.

Hardwork and perseverance worked very well for the team this semester, as there were many difficulties in completing this project. If the team were to complete this project again, team members would reach out to experts in the field affiliated with the university in order to gain useful information in a more timely manner.

Future Work

The final design will theoretically be a functional method for detection of the E6 and E7 oncoproteins in urine. Although the physical user device consisting of the test strip housing and sample dropper have been assembled and tested, the antibody-coated nitrocellulose test strip has yet to be assembled due to a lack of funding. In order to assemble this test strip, the team would need to purchase nitrocellulose paper along with all necessary antibodies to assemble the assay. . Future work will include fully assembling the final design in a manner that keeps the completed antibody strip in place and absorbent pad directly under the insertion bubble, without compromising the accuracy of the test strip. The team would develop a method of adhering the nitrocellulose strip to the bottom of the interior of the testing device casing using a form of cyanoacrylate glue. If the cyanoacrylate is found to impede the performance of the biomarker testing device, a potential solution would be to adjust the testing device casing. The testing device casing would be modified to include barriers on either side of the device to prevent movement of the test strip without the use of an adhesive.

Future tests would consist of performing biomarker strip testing to determine the effectiveness of the strip design. The results of these tests will produce data to determine a variety of factors including accuracy and earliest detection concentrations of E6 and E7. For more information on these tests see Appendices D and E.

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Appendix A: Product Design Specifications

Early-Detection Cervical Cancer Testing Team

Preliminary Product Design Specifications

Team: Georgia Hancock, Cora Williams, Mira Baichoo, Josephine Hall, Adrienne Simpson, Karina Buttram

Client: Kebron Zegeye

BME 200/300

December 15, 2021

Problem Statement:

Cervical cancer is one of the most common cancers in women and also is one of the most treatable cancers when diagnosed early [1]. Current cervical cancer screenings include routine Pap smears and occasional HPV (human papillomavirus) oncoprotein tests using laboratory techniques. A Pap smear must be performed by a medical professional, as it requires cells to be collected from the surface of the cervix and vagina. While these tests are successful at detecting cervical cancer, they are uncomfortable and not easily accessible for people in developing countries. The development of a self-collected urine sample test would increase accessibility and allow more cervical cancer screenings to be performed worldwide, which in turn would prevent many cervical cancer-related deaths.

Client Requirements:

- Small and lightweight so that the device can easily be held
- Each device will cost between $3.00 to $5.00 US dollars
- Must be non-invasive and discrete
- Created from non-toxic materials that are not biodegradable
- Accessible to women ages 13 to 60 in developing countries
- Must be able to detect cervical cancer without the use of medical professionals

1. Physical and Operational Characteristics:

a. Performance Requirements

- This device should be a comfortable and safe alternative for detecting cervical cancer biomarkers.
- It should test for the presence of cervical cancer biomarkers and notify the user of the results through a color change without the use of medical lab facilities.
- The material should be biocompatible and non toxic to the user and should not cause any infection or inflammation.
- The design should be easy to hold and made of a rigid material that is not biodegradable.
- The design should be easily stored and distributed for home usage.

b. Safety:

- This device will remain in individual packaging to maintain a sterile environment prior to use.

- It should be biocompatible with no toxic materials and not cause any infections or inflammation.

c. Accuracy and Reliability:

- This device should be able to detect cervical cancer biomarkers from a sample collected at home. It should produce at least 70% accurate results.

d. Life in Service:

- The device should be disposed of after each use.

e. Shelf Life:

- This device should be stored in sealed, sterile packaging prior to use. The device will operate in temperatures ranging from 50^{o}F-110^{o}F. It will have a shelf life of approximately 1-3 years, while remaining in a sealed package [2].

f. Operating Environment:

- The device is designed to be used by women in developing countries in a non-medical environment.
- The device will provide clear instructions to conduct the test in any setting with no other equipment necessary.

g. Ergonomics:

- The device will be small and lightweight so that it may easily be held.

h. Size:

- The device will be 3” long and 1.5” wide.

i. Weight:

- The device weighs 0.5 oz.

k. Materials:

- Materials in contact with the user during sample collection are biocompatible
- No materials used are biodegradable

l. Aesthetics, Appearance, and Finish:

- This device is compact so that it can be easily be held in the users hand
- Results should be easy to read and use no words so that users who speak any language can read the results universally
- Test should be discrete in appearance to avoid taboos around women’s health

2. Product Characteristics

a. Quantity:

- One sample collection cup
- One 3mL pipette
- One biomarker testing device

b. Target Product Cost:

- The device should cost between $3-$5 per test to manufacture.

3. Miscellaneous

a. Standards and Specifications:

- Does not require a doctor or other healthcare professionals for collection or interpretation of results
- Discrete packaging
- Clear indicator of positive or negative results

b. Patient-Related Concerns:

- This product is designed for women in rural areas who do not have access to doctors or healthcare. The test must be easy to use and available for women in cultures where women's health topics are not discussed. It is important that the product will provide a clear answer if the patient has cervical cancer markers or not, this way the patient can make an informed decision toward next steps and receive medical care.

c. Competition:

- Currently there is no non-invasive method for testing for cervical cancer. The main way for testing is a Pap Smear, which is a very invasive method, requires a doctor, and can be very expensive. A Pap Smear, is where a provider inserts a speculum to visualize the cervix and uses a wooden or plastic scraper and/or cervical brush to collect cell samples[3]. This method helps screen for abnormal cells that have the ability to turn into cervical cancer.
- The OncoE6 Cervical Test is a rapid and easy-to-use test based on the detection of E6 oncoproteins from high risk HPV types 16 and 18 using highly specific monoclonal antibodies (mAbs) in a lateral-flow (LF) assay format. It is available in the US, as a service through our CLIA-certified laboratory. This qualitative test is used to analyze cells extracted from cervical cytology swab specimens [4].
- Another method used in more rural parts of Africa and India is Visual Inspection with Acetic Acid(VIA). The procedure is similar to a Pap Smear except a 5% solution of acetic acid is swabbed onto the cervix and left there for 60 seconds. After the time has passed, a precancerous lesion will turn white with clear and dense margins, this is considered a positive result [5]. After a positive result the patient would be referred for further treatment.

References

[1] “Cervical cancer,” *World Health Organization*. [Online]. Available: https://www.who.int/health-topics/cervical-cancer#tab=tab_1. [Accessed: 23-Sep-2021].

[2] the H. E. Team, “Do pregnancy tests expire? what to know before using one,” Healthline, 16-Dec-2019. [Online]. Available: https://www.healthline.com/health/pregnancy/do-pregnancy-tests-expire. [Accessed: 12-Dec-2021].

[3] “HPV and PAP testing,” *National Cancer Institute*, 20-Dec-2019. [Online]. Available: https://www.cancer.gov/types/cervical/pap-hpv-testing-fact-sheet#what-is-cervical-cancer-screening. [Accessed: 23-Sep-2021].

[4] “OncoE6 Cervical Test,” Arbor Vita Corporation, 25-May-2020. [Online]. Available: https://www.arborvita.com/oncoe6/. [Accessed: 03-Nov-2021].

[5] U. R. Poli, P. D. Bidinger, and S. Gowrishankar, “Visual inspection with acetic acid (VIA) screening program: 7 years experience in early detection of cervical cancer and pre-cancers in rural South India,” *Indian Journal of Community Medicine*, vol. 40, no. 3, p. 203, 2015.

Appendix B: Materials and Expenses

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Mini Pipettes	small pipettes used to drop liquid into a small opening	V Cool Livat	shvk-science eye droppers	11/10/21	100	$0.05	$5.19	https://www.amazon.com/dp/B07MSNQYTV/ref=syn_sd_onsite_desktop_114?psc=1&pd_rd_plhdr=t&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUExU1M5UjdUVVVXMU1EJmVuY3J5cHRlZElkPUEwOTY4OTk1MjVISjdGSTREMTEzVCZlbmNyeXB0ZWRBZElkPUExMDQ1Nzk0MTRYMzQySFVOUDZSRyZ3aWRnZXROYW1lPXNk

								X29uc2l0ZV9kZXNrdG9wJmFjdGlvbj1jbGlja1JlZGlyZWN0JmRvTm90TG9nQ2xpY2s9dHJ1ZQ==
“TOUGH’ Resin Filament	any filament used while 3D printing	MakerSpace		11/1/21, 12/1/21	2 Prototypes	$1.06, $7.30	$8.36	
Super Glue	glue two sides of the prototype together	Super Glue	SGC SGH2		1	$2.79	$2.79	https://www.amazon.com/Super-Glue-SGH2-48-Single/dp/B00009V3VE/ref=asc_df_B00009V3VE/?tag=hyprod-20&linkCode=df0&hvadid=533605078460&hvpos=&hvnetw=g&hvrand=2017293513478683258&hvpone=&hvptwo=&hvqmt=&hvdev=c&hvdvcmdl=&hvlocint=&hvlocphy=9018948&hvtargid=pla-14340829532 70&psc=1

Appendix C: Expected Materials and Expenses

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Mini Pipettes	small pipettes used to drop liquid into a small opening	V Cool Livat	shvk-science eye droppers	11/10/21	100	$0.05	$5.00	https://www.amazon.com/dp/B07MSNQYTV/ref=syn_sd_onsite_desktop_114?psc=1&pd_rd_plhdr=t&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUExU1M5UjdUVVVXMU1EJmVuY3J5cHRlZEIkPUEwOTY4OTk1MjVISjdGSTREMTEzVCZlbmNyeXB0ZWRBZEIkPUExMDQ1Nzk0MTRYMzQySFVOUDZSRyZ3aWRnZXROYW1IP

								XNkX29uc2l0ZV9kZXNrdG9wJmFjdGlvbj1jbGlja1JlZGlyZWN0JmRvTm90TG9nQ2xpY2s9dHJ1ZQ==
“TOUGH” Resin Filament	any filament used while 3D printing	MakerSpace		11/3/21 12/1/21		$1.06 $7.30	$8.36	
Clear cups	5.0 fl oz. clear, plastic cups	Prestee	B0757YV1W1		100	$0.16	$16.00	https://www.amazon.com/Plastic-Disposable-Cocktail-Drinking-Tumblers/dp/B0757YV1W1/ref=sr_1_8?dchild=1&keywords=6+oz+clear+cups&qid=1633619425&sr=8-8
Super Glue	glue two sides of the prototype together	Super Glue	SGC SGH 2		1	$2.79	$2.79	https://www.amazon.com/Super-Glue-SGH2-48-Single/dp/B00009V3VE/ref=asc_df_B00009V3VE/?tag=hyprod-20&linkCode=df0&hvadid=533605078460&hvpos=&hvnetw=g&hvrand=2017293513478683258&hvpone=&hvptwo=&hvqmt=&hvdev=c&hvdvcmdl=&hvlocint=&hvlocphy=9018948&hvtargid=pla-1434082953270&psc=1
Anti-HPV 16 + 18 E6 antibody		abcam			100 µg	$4.45 per µg	$445.00	https://www.abcam.com/hpv16-e6-hpv18-e6-antibody-c1p5-ab70.html
Anti-HPV 16 E7 antibody		abcam			100 µg	$4.45 per µg	$445.00	https://www.abcam.com/human-papillomavirus-16-e7-antibody-tvg-701y-ab20191.html
Anti-HPV 18 E7 antibody		abcam			100 µl	$4.80 per µl	$480.00	https://www.abcam.com/hpv18-e7-antibody-8e2-ab100953.html

Recombinant HPV16 E6 protein		abcam			100 µg	$5.10 per µg	$510.00	https://www.abcam.com/recombinant-hpv16-e6-protein-his-tag-ab226447.html
Recombinant Human papillomavirus HPV18 E7 protein		abcam			100 µg	$9.50 per µg	$950.00	https://www.abcam.com/recombinant-human-papillomavirus-hpv18-e7-protein-his-tag-ab236931.html
Recombinant Human Papillomavirus 16 (E7) protein		abcam			100 µg	$5.90 per µg	$590.00	https://www.abcam.com/recombinant-human-papillomavirus-16-e7-protein-his-tag-ab237790.html
Goat Anti-Rabbit IgG H&L		abcam			500 µg	$0.28 per µg	$140.00	https://www.abcam.com/goat-rabbit-igg-hl-alexa-fluor-488-ab150077.html
Recombinant Anti-Rabbit IgG antibody		abcam			100 µl	$5.20 per µl	$520.00	https://www.abcam.com/alexa-fluor-488-rabbit-igg-antibody-sp137-ab270142.html
Universal Lateral Flow Assay Kit		abcam			100 tests	$16.60 per test	$1660.00	https://www.abcam.com/universal-lateral-flow-assay-kit-ab270537.html
Blue Polystyrene Latex Particles	0.08 µm diameter particles	Magsphere	PSB080NM		5 mL	$30.80 per L	$154.00	https://www.magsphere.com/Products/Colored-Polystyrene-Particles/colored-polystyrene-particles.html
Anti-p53 Antibody		abcam			100 µg	$4.60 per µg	$460.00	https://www.abcam.com/p53-antibody-pab-240-ab26.html
Recombinant anti-pRb antibody		abcam			100 µl	$5.30 per µl	$530.00	https://www.abcam.com/rb-antibody-epr17512-ab181616.html
Recombinant anti-UBE3A antibody		abcam			100 µl	$4.80 per µl	$480.00	https://www.abcam.com/ube3a-antibody-epr23077-14-ab272168.html
							Total: $7396.15	

Appendix D: Testing Protocols

1. Absorbent Pad Testing
    - A series of tests will be run to determine the effectiveness of the absorbent pad to slowly wick urine down the test strip.
    - Water will be dropped onto the absorbent pad of the test strip for each test and the flow rate will be recorded.
    - The maximum and minimum flow rate necessary for a valid test will then be determined by finding the maximum and minimum amount of time it takes for the control line to appear.
2. Concentration of E6/E7 in Urine
    - A series of tests will be run with urine from patients at different stages of cervical cancer to determine the concentration of E6/E7 in urine at each stage of cervical cancer progression.
3. Positive Line Visibility
    - A series of tests will be run with different concentrations of E6/E7 in water to determine the lowest concentration of E6/E7 detectable by the device.
    - The visibility of the line produced by the binding of the antibodies to E6 and E7 at varying concentrations will be recorded.
4. Multiple HPV Strain Testing
    - A series of tests will be run using cervical cancer causing HPV strains other than HPV 16 and HPV 18 to confirm that strains aside from HPV 16 and HPV 18 containing E6/E7 will also demonstrate a positive result on the nitrocellulose strip.
5. Determine if both E6 and E7 are necessary for test
    - A series of tests will be run using varying concentrations of E6/E7 to determine the consistency of the E6 and E7 positivity lines.

Appendix E: Testing Protocol Results

1. Absorbent Pad Testing
    - If the absorbent pad does not slow the flow of the liquid enough to accurately run the issue will need to be resolved by means of an incline or material change.
    - If the absorbent pad does not allow for a fast enough flow rate to get the results in a reasonable amount of time or does not allow for the liquid to flow to the end of the strip, the issue will need to be resolved by means of a decline or material change.
2. Concentration of E6/E7 in Urine
    - The average concentration of E6/E7 in the urine at each stage would be the lower limit concentration used for each stage in the device testing.
3. Positive Line Visibility

    - The concentration of E6/E7 that produced, consistently, the lightest positive line detectable by the human eye would correlate to the lowest level concentration of E6/ E7 detectable by the device.
4. Multiple HPV Strain Testing
    - If the test yielded more inaccurate and inconclusive results for cervical cancer causing HPV strains other 16 and 18 than the HPV16 and HPV18 strains, the test will need to be reevaluated to include detectable biomarkers in less common strains of cervical cancer causing HPV.
    - If the test yields equally accurate or better results, then the test can be validated for use across all cervical cancer causing HPV strains.
5. Determine if both E6 and E7 are necessary for test
    - If the test results show consistent detection of one or both oncoproteins at each stage of cervical cancer, the test can be compacted into only two test lines, the control line and one of the antibody test lines.
    - If the test results vary in the detection of each oncoprotein at each stage, then both antibody test lines will still be necessary for the test strip along with the control line.