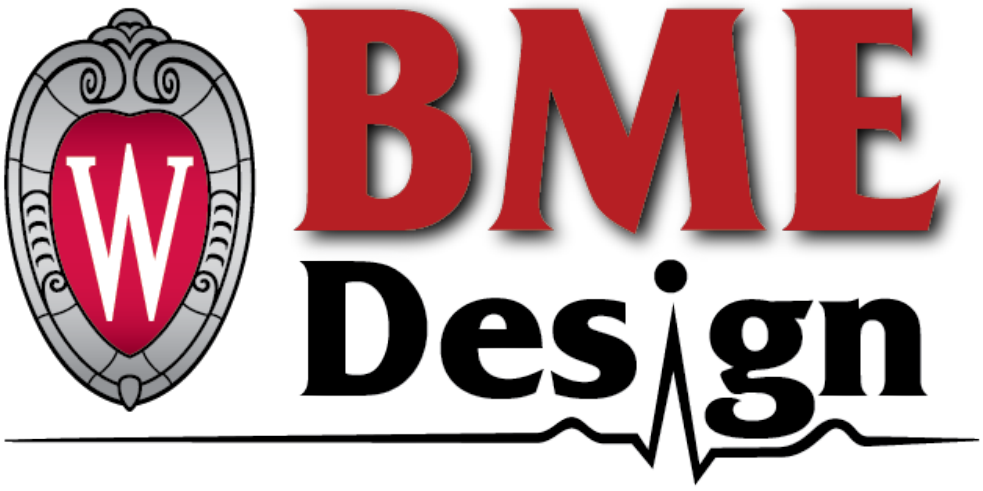

Non-Invasive Cervical Cancer Screening

Preliminary Report

BME 2/300

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October 19, 2021

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I. Abstract

Cervical cancer is one of the most prevalent cancers in women while also being one of the most treatable cancers when diagnosed early [1]. Many women do not have access to early screening options, such as the Pap smear, for various reasons, such as cost, minimal access to hospitals, and fear. The proposed device would test a self-collected urine sample for the presence of HPV. This would provide a discrete and cost effective option for women to screen for cervical cancer-causing HPV in a home environment. The device would indicate the presence of HPV through a color change in the test strip viewable through a window in the device. Further research is needed to choose the most suitable biomarker for testing. This device would allow women to have more autonomy surrounding their health and remove fear associated with invasive testing methods.

II. Introduction

Cervical cancer is one of the most treatable cancers when caught early. This type of cancer affects the lowermost part of the uterus; its cause is the long-term effects of the human papillomavirus (HPV). HPV accounts for 91% of all cervical cancer [2]. There are many strains of HPV; however, strains 16 and 18 are the two most predominant cancer-causing [3]. The Pap smear is the most common method for cervical cancer screening and is a very invasive procedure. 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 [4]. This method helps screen for abnormal cells that have the ability to turn into cervical cancer. An HPV vaccine is available that protects against the two main cancer causing strains mentioned above, and 7 other strains that have also been linked to cervical cancer and genital warts [5].

The main goal for this project is to create a non-invasive method to detect HPV in developing countries without easy access to healthcare. The top 20 countries with the highest rates of cervical cancer are within Africa [6]. The causes of this can be linked to limited access to hospitals and clinics, limited funds to pay for testing, and a cultural taboo around women's health [7]. The test will allow women to conduct the test themselves, eliminating the need to go to hospitals far away and ease the cultural taboo around women's health. The device would allow for earlier detection of cervical cancer causing HPV strains in rural parts of the world. The development of a discrete, self-collected urine sample test would increase early cervical cancer detection by providing a cost-effective and culturally-sensitive screening option for women. This device would allow screenings for HPV to be easily accessible worldwide, which in turn would prevent many cervical cancer-related deaths.

III. Background

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. Cervical cancer is one of the most common cancers in women and is one of the most treatable when diagnosed early [1]. 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].

Before designing the cervical cancer testing apparatus, research was required to determine which bodily fluid is the best for detecting cervical cancer-causing HPV strains. After completing this research, there were three potential bodily fluids for testing: blood, saliva, and urine. To see the decision making process for choosing the bodily fluid, see the Preliminary Design Evaluation section below.

After choosing the bodily fluid for testing, three designs were drafted for the testing apparatus. To see the designs and the decision making process for choosing the best testing apparatus design, see the Preliminary Design Matrix and Evaluation sections below.

The client, Ms. Kebron Zeygeye, wants the design and prototype a non-invasive cervical cancer test that does not require the sample to be collected by a medical professional or sent out to a lab for testing. An additional project requirement was for the final cost of the test for the consumer to be between $3-$5 USD so that it is easily accessible for those living in rural areas or developing countries. The test also needs to be easy to use and understand the results. The test should be made of biocompatible, non-toxic, and non-biodegradable materials. To see a more detailed project description, see Appendix 1 below for the Product Design Specifications.

IV. Preliminary Designs

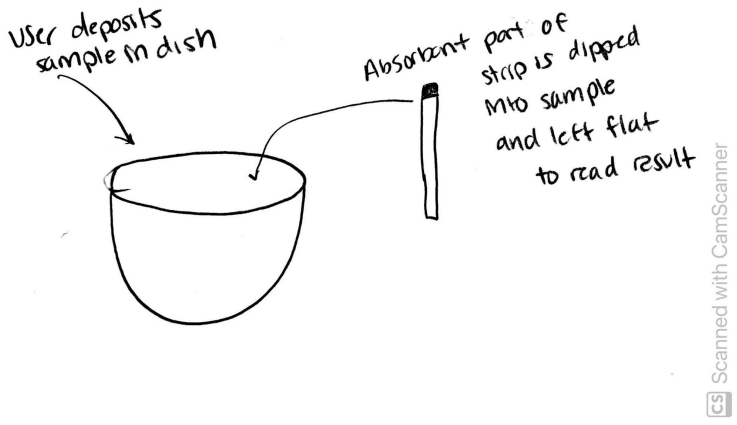

Figure 1. Strip Dip Collection Device

The first preliminary design is the Strip Dip collection device. It contains a cup that will hold the user's urine and an absorbent pad that will be dipped into the cup of urine. The intention is for the strip to give some indication (color changing or positive/negative symbols) of the results after being laid flat for a period of time to allow for the urine to react with the reagent coating the strip.

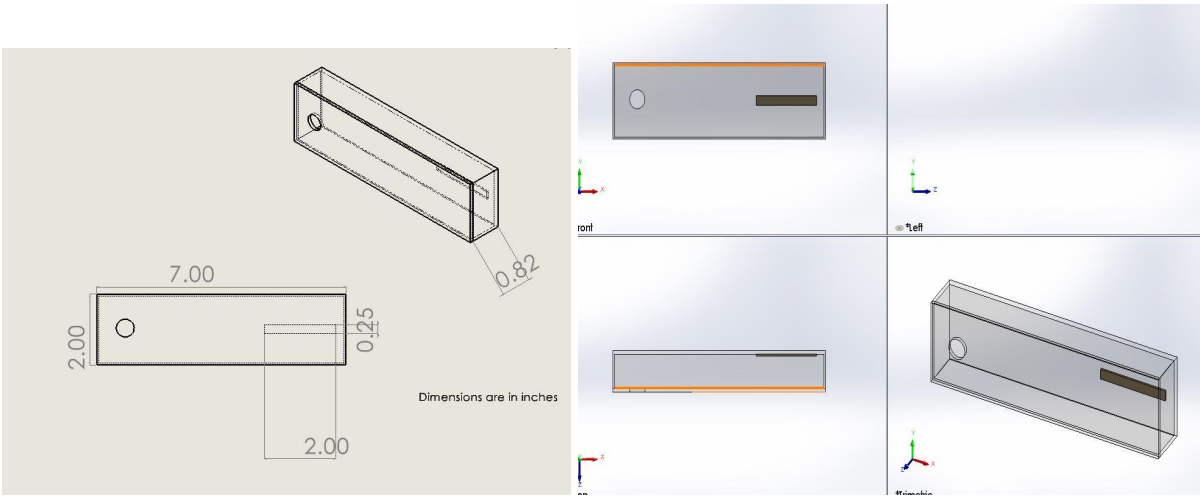

Figure 2. Drop Test Collection Device

The second preliminary design is the Drop Test collection device. It contains a dropper pipette to transfer the urine sample onto the test strip and an absorbent pad coated in a reagent and encased in a plastic covering to protect the strip. The user will drop a few drops of urine into the hole on top of the device. The absorbent pad will then carry the sample to the reagent and give some indication of the results (color changing or positive/negative symbols).

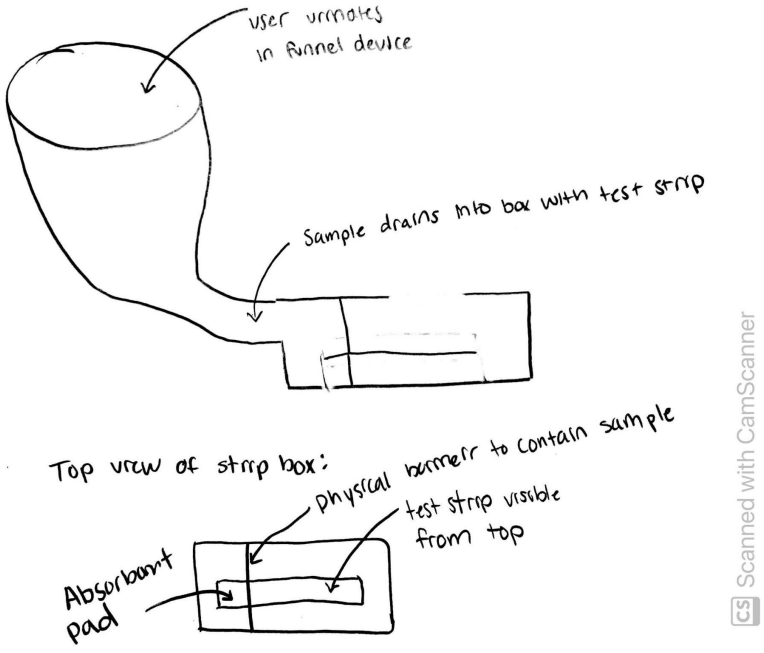

Figure 3. Funnel Device

The third preliminary design is the Funnel Device collection method. The design is similar to Drop Test design in that it contains an absorbent pad coated with a reagent and encased in a plastic container to protect the strip. The plastic container with the strip is connected to a funnel which the user urinates directly into. The urine is then carried to the front half of the absorbent strip. There is a divider between the absorbent pad and the reagent-coated strip that prevents any urine from coming into contact with the reagent side unless it is through the absorbent pad. Similar to the first two designs, the device will give the user some indication as to the results of the test.

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 highest ranked criteria was prior detection. This was to ensure adequate prior research and experimentation with the selected sample type had been done. Urine ranked the highest of the samples due to the numerous HPV detection experiments previously done. The next highest criteria is the ease of obtaining a usable sample. The sample needs to be uncontaminated to allow for the most accurate testing. Saliva ranked the lowest because there is the highest possibility of collecting contaminants in the sample. 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 use as the user must easily understand the collection requirements in order for the device to be most effective. 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 everyone. Strip dip was highest in this category because it would be the most economical to price a cup and test stick between 3 and 5 dollars. The next category was ease of fabrication to ensure the device was simple enough to fabricate and that it wouldn't require many moving parts that could malfunction or cause leakage. The highest, again, was the strip dip because the only thing needed is a cup and the test strip, neither of which are too complicated to fabricate. 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:

The materials for the design include clear cups, mini pipettes, gauze, PLA filament, and immunoassay test strips. The clear, plastic cups are intended for the user to fill with their urine sample. The user will then use the small, single use pipette to transfer a few drops of urine into

the collection mechanism. The collection mechanism will be 3D printed from PLA filament and used to encase the immunoassay test strips. The plan is to use a test strip coated in a biomarker to show the test results; however, this is subject to change. The immunoassay strip will not be directly exposed to the urine sample, so the gauze pads will be compressed into an absorbent pad that will absorb the urine sample and expose it to the immunoassay test strip.

Methods:

With a prototype designed in SolidWorks, a 3D print of the collection mechanism will be made using PLA filament. After the device is printed, the test strip will be developed. The test strip will be coated in a biomarker that is to be determined. In order for the test to show results, the test strip needs to be coated in a reagent that indicates a color change when reacting with the urine sample. Once the test strip is coated, it will be placed in the small divot in the printed collection device. Several sheets of gauze will be manually compressed into a pad that will be used to absorb the urine sample. The absorbent gauze pad will be used to gradually transfer the urine sample to the end of the test strip by placing the gauze pad in the same divot as the test strip. The cup for collecting urine and pipettes for transfer urine do not require any fabrication.

Testing:

To test for potential device leakage, water will be dropped into the collection device using a pipette to simulate a urine sample. The device will then be observed to determine whether the water gets absorbed into the gauze pad. The test strip does not need to be in the collection mechanism to test.

Testing for false positive and negative results will include testing with water and with an additional substance that theoretically should react with our intended biomarker. After being coated in a biomarker and color indicating reagent, we will make the control group water. Exposing the test strip to water should not indicate any sort of color change or reaction on the test strip. We will then expose the test strip to a substance that theoretically should react with our intended biomarker to determine if the test strip will show accurate, positive results. The testing of the test strip accuracy will be performed a minimum of 10 times with many different samples and statistically analyzed to ensure accurate results. The test strip should be able to show accurate positive or negative results roughly 70% of the time. All of this testing will be performed in a room temperature, laboratory setting, following proper protocols for working with human specimens. The results of the above testing will suggest any necessary changes to the design of the collection device, the chosen biomarker, and color-indicating reagent used. Reference **Figure 2** for final prototype used for this testing.

VII. Results

In the product, the main source of error will be from the test strip color change. The test strip's main goal is to tell the user if their results are positive or negative for HPV. The way the test will do this is through a color change. The strip will be white in color, and a change to a determined color will signify a positive result, meaning the user does have HPV. If the strip stays white, and there is no color change, that is a negative result. The instances of false positive and negative results from the test strip should be minimized. A false positive would mean the strip changes colors, but the user does not have HPV. Additionally a false negative would mean no color change, but the user does have HPV. A false negative result can happen if the test is taken within the inoculation period of the virus. This is the period from when the user was first infected with HPV to when the test can accurately test for the virus. To help reduce the number of false negatives, the test should be taken every 6-12 months or more[9]. This will be feasible since the test will be around $3-5 USD, and can also be self-administered.

VIII. Discussion

Preliminary research has led the team to focus on HPV antibodies and the HPV E6 oncoprotein as potential biomarkers for HPV and cervical cancer. Urine has previously been used to detect cervical cancer but often requires the use of laboratory techniques such as methylation and DNA sequencing [10]. HPV antibodies and HPV E6 oncoproteins could potentially be tested in a home setting and not require laboratory analysis, fulfilling a primary objective of the design requirements. However, both of these potential biomarkers require further research to determine the full parameters necessary for detection. Studies have shown that there is a significantly higher concentration of HPV antibodies found in first void urine of vaccinated women vs unvaccinated women [10,11]. Further research on the concentration of HPV antibodies in HPV positive and negative women regardless of vaccination status is necessary to determine if the antibody concentration levels are significantly different enough to be used for detection. HPV E6 oncoproteins were detected in only 21% of all HPV urine samples[10,12]. However, this oncoprotein was detected in 52% of urine samples positive for invasive cervical cancer [11,12]. This oncoprotein is best found for more invasive strains of HPV with a higher risk of cervical cancer. These markers have the potential to be useful in HPV and cervical cancer detection, but require an increased understanding of the stage of infection when the biomarkers appear, their concentration levels, and where in the urine they can be found.

IX. Conclusions

Summary

The team was tasked with exploring the possibility of testing for early signs of cervical cancer via an at-home point of care diagnostic device targeted towards women in rural Africa. The team sought out to not only create a physical testing device to be used for this process, but also to compile research on useful biomarkers that could detect HPV without the use of expensive lab technology. The goal for this project was to create an at-home test that would be between $3-$5.

After meeting with Ms. Zeygeye and discussing her requirements, the team began research and decided to create 2 matrices for decision making to ensure success of the project moving forward. The first matrix ranked 3 different sample types: blood, urine and saliva, in 5 categories. Urine was chosen as the sample for the test due to its ease of collection and a significant amount of research proving that HPV strains 16 and 18 could be detected in it. The team then moved on to brainstorming designs for a mechanical device used for testing.

The first design, “strip dip”, consists only of a dish for the user to collect the urine sample in as well as the strip used for testing. In this simplistic design, the user would simply immerse the absorbent square of the test strip into the sample and lay it flat to read the result. Design two, the “drop test”, featured a low-profile box in which the testing strip would sit, featuring a hole over the absorbent pad where the user could accurately deposit the sample directly onto the absorbent pad, allowing the sample to deposit onto the strip and for the result to be visible to the user from a transparent cutout in the top. Design three, the “funnel device”, was a similar system to the drop test but instead of manually depositing the sample onto the strip, the user would simply urinate through the funnel which would feed the sample to the device. After putting these ideas through the design matrix, the drop test was chosen as it is compact, cost-effective, and gives little room for user error while maintaining ease of use.

Future Work

Now that the preliminary design for the physical device has been created, the team will start the prototyping process, starting with 3D-printing the model for a durable, plastic prototype. Other materials including droppers will be purchased to test the prototype’s usability. Additionally, further research will be conducted on theoretical testing possibilities including which biomarker will be chosen as well as what it binds with to determine how the chemical properties of the test could function.

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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 300/200

September 24, 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 Pap smears and HPV (human papillomavirus) tests. Testing methods such as the Pap smear must be collected by a medical professional, as it requires cells to be collected from the surface of the cervix and vagina [3]. While these tests are somewhat successful at detecting cervical cancer [2], they are not easily accessible for people in developing countries and can be an uncomfortable experience. The development of a discrete self-collected urine sample test would increase early cervical cancer detection by providing a cost-effect and culturally sensitive screening option. This device would allow cervical cancer screenings to be easily accessible 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 HPV markers.
- It should test for the presence of certain HPV strains and/or cervical cancer biomarkers and notify the user of the results 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 HPV markers 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 year, while remaining in a sealed package.

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 needs to be as small as possible without compromising the usability of the device and should be no larger than 10” long and 5” wide.

i. Weight:

- The device should be lightweight to increase usability and to prevent unnecessary stress on the user and should weigh no more than 0.5 pounds.

k. Materials:

- Materials used for the sample collection method should be biocompatible
- No materials used should be biodegradable
- Materials should be lightweight and comfortable for the user if contact with skin is necessary
- The surface in which the sample is tested on should be capable of containing the sample for the duration of the test.

l. Aesthetics, Appearance, and Finish:

- This device should be 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 functional sample collection prototype

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
- 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 HPV 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.

- 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[4]. After a positive result the patient would be referred for further treatment.

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