Point-of-Care Human Papillomavirus Diagnostic Refinement

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***Abstract*—Point-of-care (PoC) testing requires the user to have all testing and evaluation equipment available on-site. The current PoC assay that has been developed for detection of Human Papillomavirus (HPV) requires 16 individual steps and several hours to process. This process is not suitable for deployment to the field because it requires extensive lab equipment and training. This technology will be streamlined in complexity so that it can be used by someone with minimal training in third world countries.**

Keywords: Point-of-care diagnostics, Lateral flow assay, Nano-particle, Nitrocellulose membrane, HPV detection

I. Introduction

Point-of-care (PoC) diagnostics have become an effective tool for medical testing in low resource environments and austere locations. PoC diagnostics must be simple to use and rugged in their design to be effective for field use. Cancer causing HPV is not unique to any country or socioeconomic status. In order to combat this threat it is imperative that HPV can be detected in its early stages and in this case, it must be done inexpensively to cater to low resource areas. Our diagnostic assay meets the requirements for cost but requires a more streamlined protocol for test running. In its current iteration the testing strip has thirteen steps before the results can be read and evaluated by a scanning device.

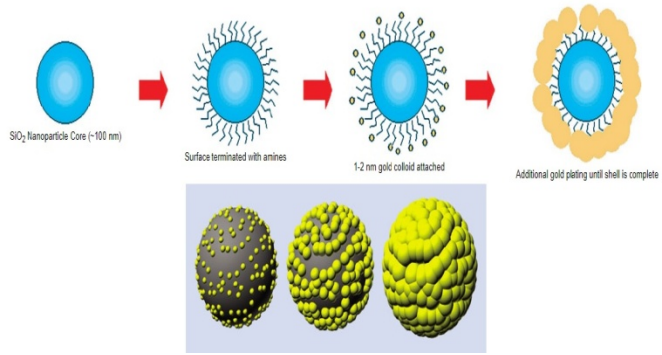

Figure 1: Gold nano-shell synthesis [3]

Currently, the PoC diagnostic's protocol takes around two hours to complete. This time estimate also relies on the test taking place in a modernized lab with an excess amount of supplies. The current assay uses nitrocellulose that is designed for quick flow rates but creates tests that are not as sensitive. We are going to implement nitrocellulose that is designed to run slower but that should require less washing steps to prevent non-specific binding and simplify the protocol. Using more suitable nitrocellulose will increase the accuracy of the tests while simultaneously decreasing the number of steps. In the current protocol there are multiple individual wash steps that require long periods of time to allow the fluid to pass through the test completely. [1] The initial pre-wetting step takes around twenty minutes before the diluted plasma serum can be applied to the sample pad. Nitrocellulose that has a smaller pore size should allow us to decrease both the number of wash steps and the amount of liquid applied per step. For our purposes we are going to use gold nano-shells as opposed to gold nano-particles. The main difference between the two gold particles is that instead of being completely gold, the nano-shells have a silica core wrapped in gold particles. This creates a less dense product that should flow faster through the assay displaying results more quickly. Using these different products, we will attempt to create an assay that is more user friendly and that meets the requirements of PoC.

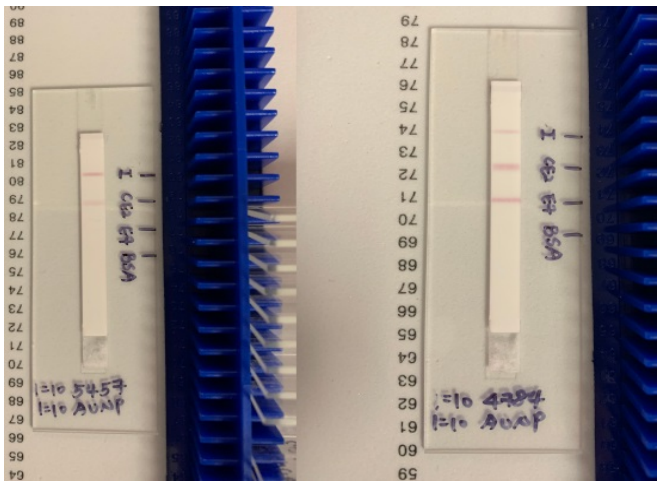

Figure 2: Examples of assays: left assay is positive for CE2 protein and right is positive for both CE2 and E7 HPV proteins.

II. Methods

A. Filter-less Scanner Calibration

Our filter-less scanner had not been used to run assays yet at the beginning of the project so the first step we took was to calibrate the scanner to work with colorimetric assays. We used both blank calibration strips and test strips using food coloring to imitate positive test results. This was accomplished by varying the capacitance linked to each test site and adjusting the intensity of the organic light emitting diodes (OLEDs) at each test site. Originally the box was fitted with one picofarad capacitors at each of the test sites. When we ran the blank calibration slide the test took around 3 seconds. We determined that was too fast to be useful for collecting sufficient data and observing the data's slope. We removed the one picofarad capacitors and replaced them with one microfarad capacitors to see if the longer charge time would help us to extend the time each test site took. With one microfarad capacitors it took too long for each site to charge and extended the time each test site took to run far past the time constraints we had set. To run the test

optimally and collect more data that showed a gradual build in voltage over time, the box was fitted with eight different capacitors ranging from 100 picofarads to 474 nanofarads. The OLEDs were also set to different intensities to adjust how quickly the light was able to penetrate the test strips and be absorbed into the photodiodes on the other side. Each OLED has to be adjusted individually because of the variance in light production and reading operation.

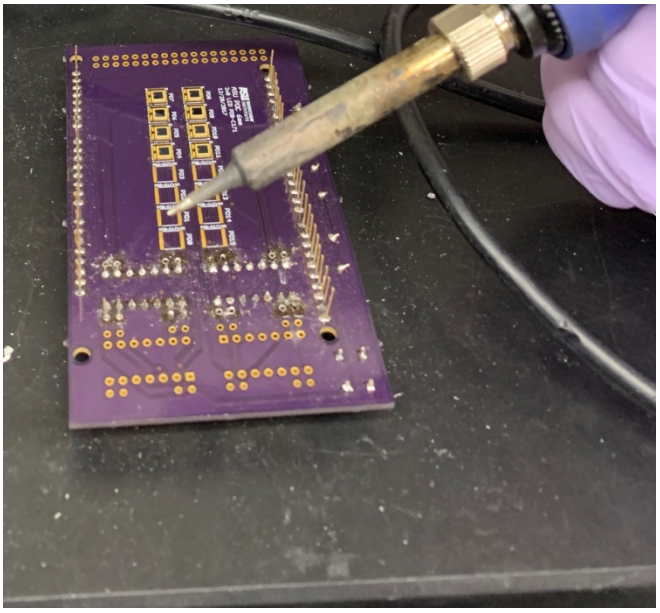

Figure 3: A variety of capacitors being soldered to the board of the filter-less scanner.

B. Food Coloring Mock-Up Slides

To be able to test how the scanner works with different colors and concentrations at the test sites we created food coloring mock-up slides with red and blue food coloring. These colors were chosen because the different indicators we were using showed up as red or blue-green to the naked eye. Mixtures of food coloring and water were prepared to the following dilutions: 1:1, 1:10, 1:100, 1:1,000, 1:10,000,1:100,000, and 1:1,000,000. The mixtures were applied to the sample end of the test strips and were allowed to flow through the strip until completely saturated. They were then placed on a hot plate to ensure that they were fully dried before placement into the scanner. They were tested using the original capacitance and OLED intensity settings. First, a blank calibration strip was placed into the scanner to ensure that each site was functioning properly; then the different dilution strips were inserted into the scanner and allowed to run to completion. The scanner uses four different test sites per strip when running from start to finish. Due to the amount of time some of the darker strips took to run, they were tested individually from site to site. All of the calibration strips were run using the right strip due to scanner limitations. The scanner uses four different test sites per strip when running from start to finish with a full set of capacitors and functioning test sites..

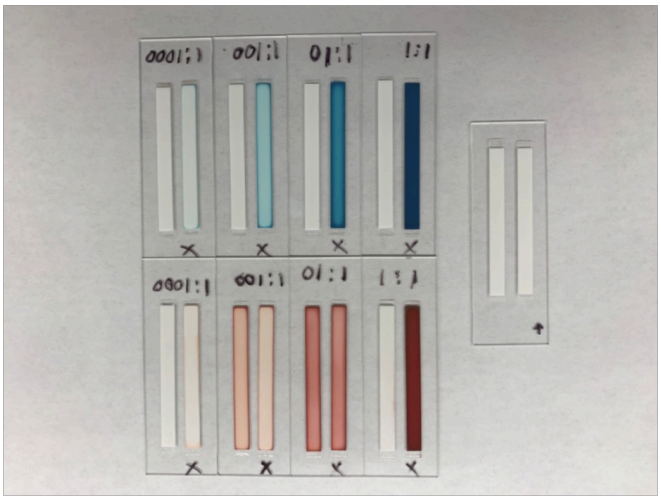

Figure 4: Mock-up food coloring slides prepared for filter-less scanner calibration.

C. Cartridge Design for Field Use

For field use, running the HPV assays on glass slides in a controlled environment would not meet the user-friendly and rugged requirements of a PoC diagnostic. In the interest of facilitating these needs, a prototype cartridge needed to be designed to contain the test strips and allow for more ease of use. The cartridge was meant to be printed using a 3D printer to allow for rapid prototyping and some level of ruggedness in design. To print these cartridges we decided to use a stereolithography (SLA) printer to minimize the porosity of the material. We also decided to use clear resin for the bottom portion of the cartridge allowing the test strips to be directly applied while still being able to use the scanner to run the finished tests. The lid uses black resin to maintain some separation between the test sites and allowing the passage of light to be directed to the photodiodes on the scanner board. They also had to be designed in a way to accommodate the amount of liquid used in each test and ease of application of patient serum.

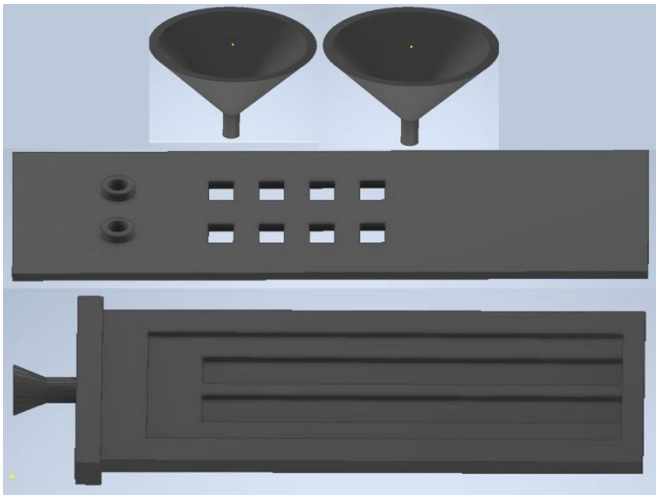

Figure 4: 3D render of the assay cartridge prototype.

D. Material Review

One of the most important parts of product development is assessing the different materials available to you and figuring out what would work best for your requirements. To accomplish that we extensively reviewed literature to learn about the different types of Point-of-Care diagnostics, and how they were designed. The main focuses were the nitrocellulose strips that allow the fluid to flow throughout the test and the nano-particles that provide an indication of a positive or negative test. For this project we proposed that nitrocellulose strips that have a slower flow rate but a higher level of sensitivity would enable the reduction of wash steps in the assay protocol. The wash steps are the most time-consuming steps and make up a majority of the process [1]. To assess the variety of nitrocellulose available, we obtained a sample pack of sheets from Sartorius Stedim Biotech. The nano-particles that were selected for testing were the Bioready gold nano-shells by Nanocomposix. These were selected for their reduced density and ability to flow through the smaller pore sizes of the slower nitrocellulose.

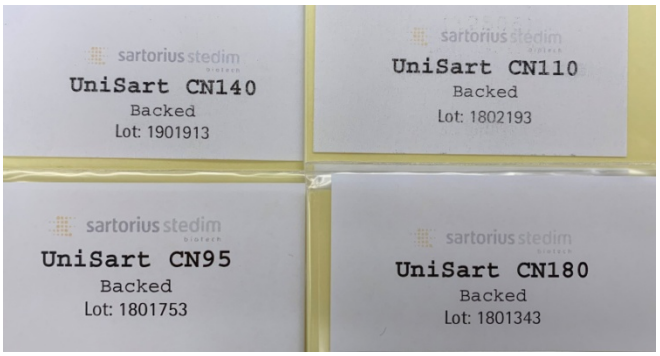

Figure 5: Variety of nitrocellulose obtained in sample pack..

III. Results and Discussion

First on the list of priorities was the calibration of the filter-less scanner. After using the before mentioned methods to test different capacitance combinations and OLED intensity settings it was determined that 82 nanofarad capacitors gave the optimal charge time without causing the test sites to run exceedingly long. At the beginning of the calibration process the scanner ran the darkest dilution test strips at an average of 2 hours per site. After running the tests with multiple set-ups the final test sites took, on average, one minute per site. The optimal dilutions chosen to represent actual test strips were 1:10 and 1:100. The 1:1 dilution was far darker than any of the actual test strips that were run and the 1:1000 dilution and even further dilutions were closer to blank test strips. The test strips were run sequentially as well as site by site with a minimal amount of variance in time taken to run the tests. After completing the testing using the food dye strips we used positive HPV test strips to ensure that the scanner would work with real-world results as well. The HPV test strip run times were in agreement with the timing we received from the food dye

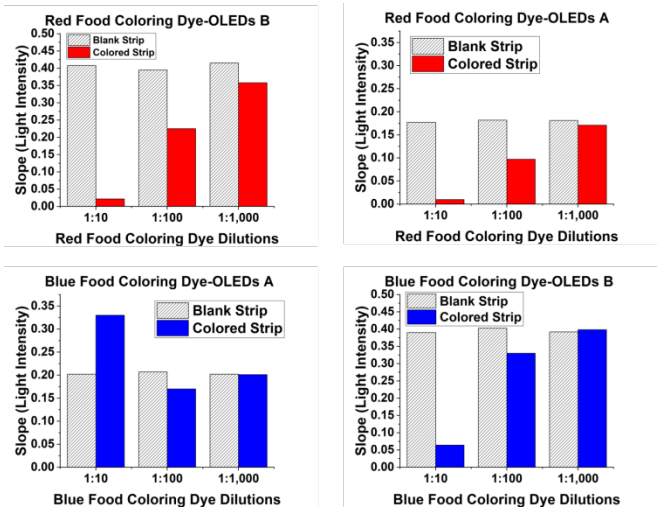

Figure 7: Graphs depicting results of food coloring calibration

strips. The darkest of the positive test strips took around 50 seconds to run versus the blank calibration strip which was set to take 10 seconds to run. The next step in the process was to develop a 3D printed cartridge to hold the test strips and survive austere conditions. The most recent functional prototype features a clear cartridge to hold the test strips and associated liquids, a clear lid with two entry points to allow for ease of sample addition, and two funnels that fit into the entry points to prevent the loss of sample fluid due to user error. The resulting print allowed for nitrocellulose strips to be laid directly on the cartridge with alignment between the openings on the lid and the visible positive test sites. The funnels printed nicely with a 10mm diameter, however the lower portion that fits into the lid proved to be somewhat brittle and required handling with care.

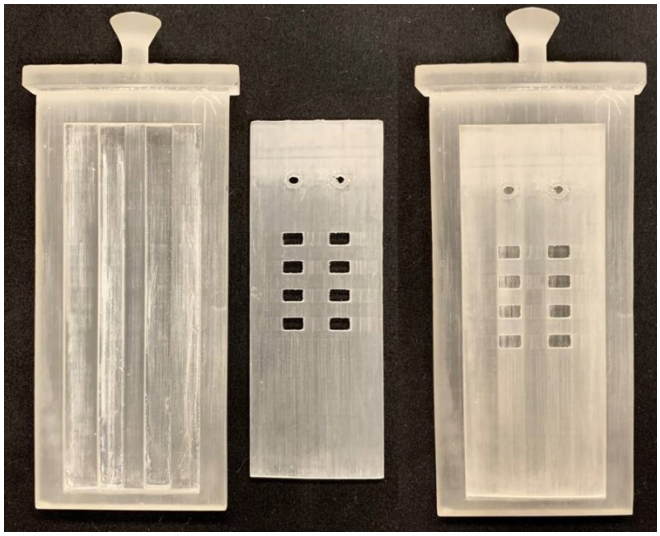

Figure 7: 3D printed assay cartridge without funnels.

The cartridge was also tested using an LED on a breadboard controlled by an Arduino to examine how the light passes through the clear resin as opposed to the glass slides that were previously used to run the assays. The light passed

through the entire cartridge easily but in comparison to glass slides there was a higher level of light pollution between the test sites. With more post-processing the resin should be able to be made more clear and allow for less overflow of light.

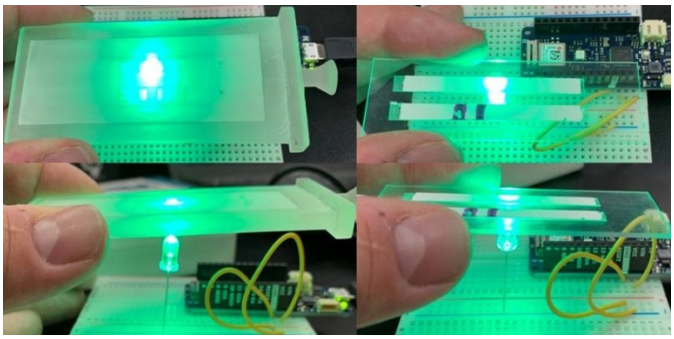

Figure 8: Light testing both the 3D cartridge and standard glass slide assay.

IV. Future work

In the future, there is still a lot of work to be done to allow the diagnostic to truly be PoC. The different nitrocellulose that was ordered will need to be tested to see how the flow rate affects the amount of non-specific binding at the test sites and the wash steps will have to be reevaluated accordingly. As for different nano-particle indicators, the nano-shells will have to be tested to see how they interact with the proteins as well as with the new variety of nitrocellulose. All of the new strips will also need to be run using the updated scanner to ensure that the proper amount of data is collected per test site. While the scanner has been calibrated to run more quickly there still needs to be more testing with real positive and negative HPV samples to determine what the level of accuracy is at each test site. The structure of the scanner also needs to be adjusted to focus the light more on each individual test site as the accuracy of the test site printing is improved. Adjustments to the cartridge lid will have to be made to ensure that the test site openings are in alignment with the structure of the scanner. Continued rapid prototyping of the entire cartridge model will be needed to make the cartridge meet all dimensional requirements and also to ensure for proper containment of liquid as the protocol to run the assay is evolved. The funnels in particular will need to be made more robust to allow untrained personnel to handle them without having to be worried about structural failure. We will be studying different post-processing methods for the SLA prints to allow them to be made more clear, closer in resemblance to the glass slides than their current form.

V. Conclusion

In this paper we have shown significant progress in making the Human Papillomavirus lateral flow assay quicker and more user friendly per Point-of-Care requirements. The end goal of this research is to develop an assay that can be deployed into the field where untrained workers can run the assays without the direction of medical staff and allow for low resource areas of the world to have access to medical diagnostics for cancer causing HPV. We have demonstrated great strides in making his a reality and with the development of the self-contained cartridge assay we have shown that it is possible to make the test more rugged and suitable for austere environments.

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