Rapid Assay for High-Risk HPV Detection

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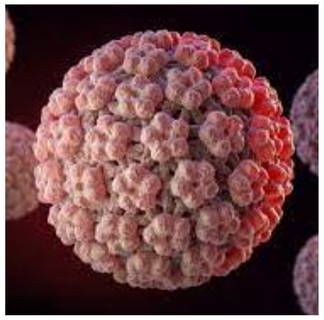
ABSTRACT

Human papillomavirus (HPV) is an extremely common sexually transmitted infection (STI) that can lead to cervical cancer. A rapid-diagnostic test, similar to an in-clinic COVID-test, is imperative to testing communities and devising a treatment plan for communities underserved by traditional cervical cancer screening. The goal of the project is to develop a rapid test that can determine if someone is HPV positive with a high sensitivity, affordability, and requiring little to no special equipment. One method used by this test is recombinase polymerase amplification (RPA) which copies DNA at an affordable cost. RPA is a form of isothermal nucleic acid amplification meaning that it can copy DNA with minimal laboratory equipment, and therefore could be performed in an outpatient clinic or home on an inexpensive rapid test. I have been optimizing the RPA assay for sensitivity and adapting it to display results on a user-friendly lateral flow strip format. So far, we have established a limit of detection of 1000 HPV16 DNA copies per 50 µL reaction.

Overall Project Background and Goals:

- Started with interviewing clinicians who did cervical cancer screening to understand their knowledge of rapid testing and user needs of a rapid diagnostic test.
- Based on the user needs, we have been designing a rapid test for HPV
- Goal 1: Amplify the HPV16 DNA so the test can determine if someone is HPV positive
- Goal 2: Add the amplification procedure onto a paper based rapid diagnostic test (similar to a COVID-19 test)



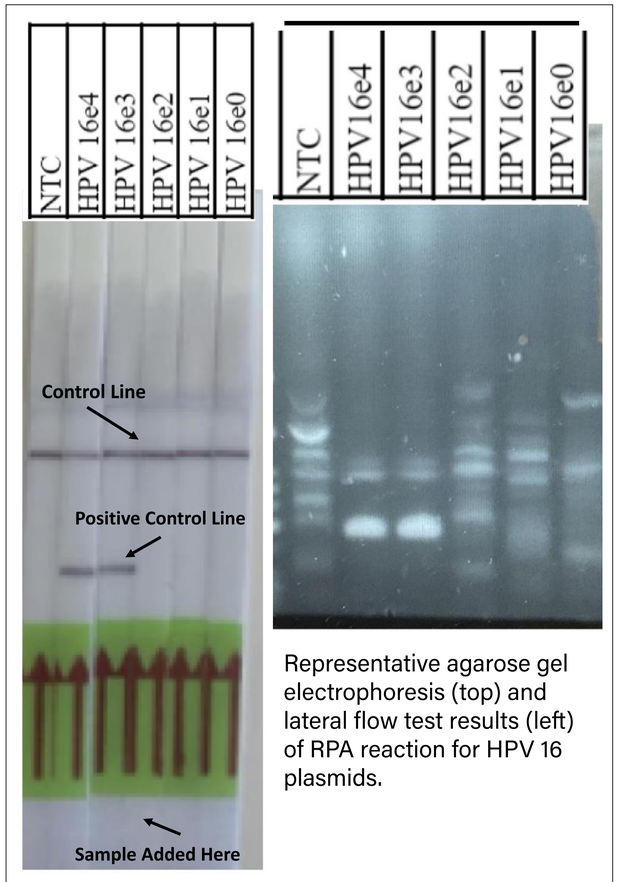
HPV Biology and Cervical Cancer

- How to prevent cervical cancer
  - Vaccinated against HPV
  - Catch infection at an early stage.
- In lower income communities, there is limited access to preventive and screening measures.

- HPV is a common STI that is spread through sexual contact
- In 2020, cervical cancer was the fourth most common cancer in women globally (WHO).
- Two strains of HPV, low risk and high risk.
  - High risk: can develop into cancer
  - Low risk: most people recover, cannot cause cancer
  - HPV 16 and 18, account for ~ 50% of cervical pre-cancers.

RPA

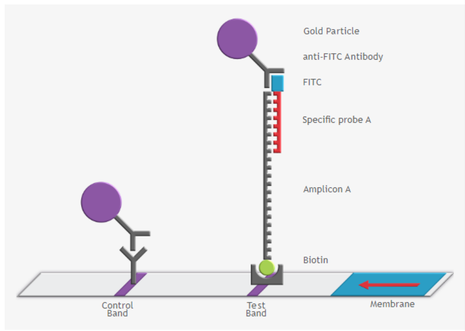
- Gong et al 2021. proposed a new way to test for HPV by combining RPA with CRISPR-Cas12a technology.
- Can be completed in 35 minutes with a high sensitivity (500 copies/reaction) (Gong).
- RPA performs the same procedure as qPCR, however, it is isothermal which means the temperature is constant for the entire reaction.
  - Faster run time and a less costly test for the patient.



Representative agarose gel electrophoresis (top) and lateral flow test results (left) of RPA reaction for HPV 16 plasmids.

Limit of Detection Results

- No positive line in the NTC which implies no contamination in the samples and that nothing extra reacted.
- As the copies decrease, the positive line becomes fainter and fainter.
- This gives the impression that the test will have a low limit of detection which means that even at low concentrations, the test can detect the HPV DNA.
- The limit of detection is 1000 HPV copies per 50 uL reaction (n=3).



Lateral Flow Strip

- Double labeled amplicons
  - One end has a fluorophore and the other has a protein that binds to the gold nanoparticle on the paper assay which creates the test lines.
- 1 uL of RPA mixture was added to the paper test where the mixture bind to the gold nanoparticles and traveled up the assay.

Next Steps:

- Optimize reaction to perform without shaking
- Test with synthetic vaginal fluid
- Perform on a paper substrate

Citations:

Gong, J., Zhang, G., Wang, W. *et al.* A simple and rapid diagnostic method for 13 types of high-risk human papillomavirus (HR-HPV) detection using CRISPR-Cas12a technology. *Sci Rep* 11, 12800 (2021). https://doi.org/10.1038/s41598-021-92329-2
World Health Organization. (2022, February 22). *Cervical cancer*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/cervical-cancer

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