Poster 32: Simplification of Molecular Diagnostics for Point-of-Care HPV Screening

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Topic: Translational Research

Objectives

HPV-associated cancers represent a significant global health burden, accounting for 5% of malignancies worldwide. An estimated 630,000 new HPV-associated cancers are reported annually, with cervical cancer being the most common. Low-income regions, particularly Sub-Saharan Africa, Latin America, Eastern Europe, and Southeast Asia, are disproportionately affected with the highest rates of cervical HPV infection. These regions often lack adequate access to sensitive point-of-care screening tools, preventing timely diagnosis and treatment. Current low-cost methods, such as Pap smears and visual inspection with acetic acid suffer from low sensitivity, specificity, and reproducibility which limit their effectiveness as a screening modality. Many countries use nucleic acid amplification tests (NAATs) which offer rapid and accurate results. Due to the expenditure of NAAT-capable equipment and reagents, however, they are often cost-prohibitive for rural and underserved communities. Our objective was to develop a novel simplified, point-of-care isothermal HPV molecular test that can detect all 14 cancer-causing HPV strains combined with G-quadruplex DNAzyme colorimetric assay for visual detection.

Methods

We developed a novel isothermal amplification technique called Stem-loop Amplification (SLA) related to loop-mediated isothermal amplification (LAMP), which uses modified looped primers to enable exponential amplification at 60C for 1 hour. SLA only targets two binding sites compared to 4-6 in LAMP, simplifying assay design. To detect the amplified products, we used G-quadruplex peroxidase-like DNAzyme probes which reduce ABTS to produce a visually interpreted result. Optimization strategies and analytical validation to evaluate the method's limit of detection, specificity, and accuracy were performed. As proof of concept in a relevant matrix, clinical cervical brushing collected in ThinPrep Pap media were tested with this method and compared to an FDA-approved reference qPCR test.

Results

Repeated experiments demonstrate that the lowest detectable HPV DNA concentration with this method is approximately 1,000 genomic copies/uL with no amplification of other pathogens. The addition of Bst 3.0 polymerase increases amplification efficiency and overall assay performance. The resulting amplified products were correctly detected using the G-quadruplex colorimetric method (Figure 1). Current investigation is focused on the improvement of signal-to-background and clinical comparison using clinical samples.

Conclusions

The application of our assay is intended as a near-patient screening tool with further evaluation by a clinician for confirmation. With an accurate and rapid screening tool, we envision low-resource regions across the world will have the tools to easily detect and treat cervical cancers in their early stages.

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