

Electrochemical Biosensing of HPV: From Molecular Detection to Clinical Impact

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Summary:

Persistent infection with high-risk human papillomavirus (HPV) is the primary cause of cervical cancer and is also associated with anogenital and head and neck cancers. HPV testing improves diagnostics but remains costly and complex. Electrochemical (EC) detection offers a low cost user friendly alternative with minimal sample consumption. Combined with loop mediated isothermal amplification it enables sensitive and fast analysis. We developed EC biosensing platforms for HPV DNA and RNA detection in cervical precancerous lesions and we currently extend this approach to urine, blood and oral samples.

Keywords: HPV, cancer, biosensor, electrochemistry, LAMP

Background, Motivation and Objective

Persistent infection with high-risk human papillomavirus (HPV) is a primary etiological driver of cervical cancer. Incorporating HPV testing into screening programs has significantly improved the effectiveness of cytological diagnostics [1]. Beyond cervical cancer, HPV is also implicated in a range of anogenital malignancies as well as head and neck cancers [2]. Current HPV detection methods often rely on costly reagents, sophisticated instrumentation, and trained personnel, limiting their broader clinical implementation. Electrochemical (EC) detection approaches offer a compelling alternative, enabling rapid analysis using compact, low-cost, and user-friendly devices. When integrated with isothermal amplification strategies such as loop-mediated isothermal amplification (LAMP), these platforms achieve high sensitivity and reduced assay time. In our work, we have developed a series of EC biosensing platforms coupled with LAMP for the detection of HPV in cervical samples from patients with precancerous lesions, demonstrating their potential for rapid and accessible molecular diagnostics [3-6].

Description of the New Method or System

We employed LAMP for the rapid amplification of HPV nucleic acids (30-40 min) from the two most oncogenic genotypes, HPV16 and HPV18. The reaction was performed in the presence of biotinylated dUTP nucleotides, enabling affinity-

based handling of the amplicons. The LAMP products were then hybridized to sequence-specific capture probes immobilized on magnetic beads, allowing efficient separation and target enrichment. EC readout was then performed using amperometric detection based on a peroxidase-mediated reaction, providing a sensitive and quantitative signal for HPV detection.

Results

Our group has reported a series of rapid and simple EC-LAMP assays for the detection of HPV16/18 in cervical cell lines and clinical smears from women with precancerous lesions. Following optimization [3], the method was validated on 61 clinical samples and benchmarked against PCR-based techniques and the INNO-LiPA genotyping assay, achieving specificity and predictive values above 90% [4]. The workflow was further streamlined by eliminating DNA extraction, enabling direct analysis of crude lysates prepared by simple heat treatment, which reduced assay complexity and minimized contamination risk [5]. More recently, we developed an RNA-based electrochemical biosensing platform that integrates reverse transcription LAMP (RT-LAMP) with rapid and simple EC readout [6]. Such diagnostics, capable of distinguishing between episomal and integrated HPV infections, can provide valuable insight into the risk of lesion progression. Our platform, termed eRT-LAMP, targeted the electrochemical detection of two key

viral mRNAs, E2 and E7. E2 is normally expressed at physiological levels, where it regulates E7 and helps prevent excessive cell proliferation. However, when the virus integrates into the host genome, the E2 gene is frequently disrupted or deleted at the integration site, resulting in loss of its regulatory role. This leads to uncontrolled E7 expression, pushing host cells toward a malignant state. The ratio of E2 to E7 expression could thus be an important biomarker for assessing the status of HPV infection. We measured these levels in cervical samples containing HPV16 using our EC-based method (Fig. 1).

Very recently, we have shifted our focus toward HPV analysis in urine samples from women with cervical precancerous lesions as a noninvasive alternative to conventional sampling. This approach is supported by studies suggesting a possible concordance between HPV detection in urine and cervical smears, indicating that urine may represent a promising and patient-friendly specimen for HPV diagnostics [7]. Our results demonstrate that HPV16 in patients is readily detectable in cervical smears, however viral loads in urine were considerably lower (Fig. 2). Finally, we have expanded our focus to the analysis of HPV in patients with oropharyngeal carcinoma (OPC), where HPV has been shown to play a critical role in tumor development and is associated with distinct clinical outcomes. In this study, we will compare oral rinse, oral swab, and plasma samples for the detection of HPV infection, evaluating their utility as prognostic biomarkers in OPC.

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Illustrations, Graphs, and Photographs

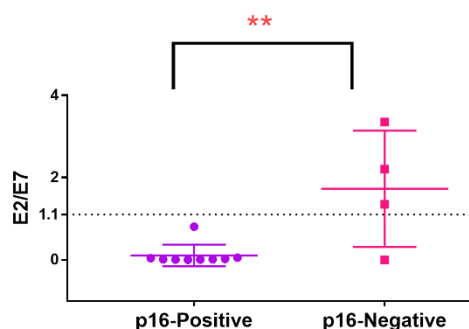


Fig. 1. Scatter plot for HPV16 comparing our EC signals (shown as E2/E7 current ratios obtained from two distinct mRNAs, left) with immunohistochemical analysis of p16. After HPV integration into the genome, E2 levels drop and E7 levels rise. This correlates with p16 positivity and disease progression. Adapted from reference [6].

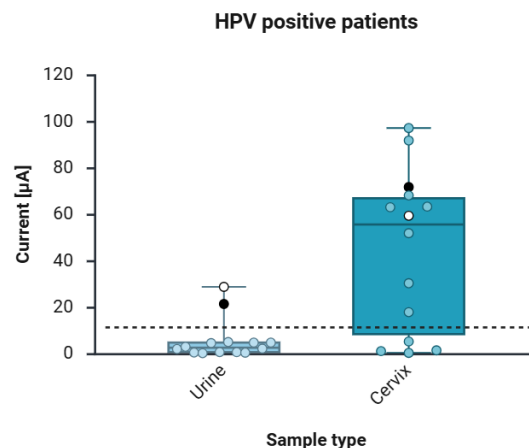


Fig. 2. Preliminary data on HPV16 detection in DNA extracted from paired samples - urine and cervical swabs - of 14 HPV16-positive patients.

References

- [1] E. T. H. Fontham et al., Cervical cancer screening for individuals at average risk: 2020 guideline update from the American Cancer Society, *CA Cancer J Clin* 70, 321-346 (2020); doi:10.3322/caac.21628
- [2] M. Lechner, J. Liu, L. Masterson, T. R. Felton, HPV-associated oropharyngeal cancer: epidemiology, molecular biology and clinical management, *Nat Rev Clin Oncol* 19, 306-327 (2022); doi: 10.1038/s41571-022-00603-7
- [3] M. Bartosik, L. Jirakova, M. Anton, B. Vojtesek, R. Hrstka, Genomagnetic LAMP-based electrochemical test for determination of high-risk HPV16 and HPV18 in clinical samples, *Anal Chim Acta* 1042, 37-43 (2018); doi: 10.1016/j.aca.2018.08.020
- [4] M. Anton, L. Moranova, R. Hrstka, M. Bartosik, Application of an electrochemical LAMP-based assay for screening of HPV16/HPV18 infection in cervical samples, *Anal Methods* 12, 822-829 (2020); doi: 10.1039/C9AY02383F
- [5] N. Izadi, R. Sebuyoya, L. Moranova, R. Hrstka, M. Anton, M. Bartosik, Electrochemical bioassay coupled to LAMP reaction for determination of high-risk HPV infection in crude lysates, *Anal Chim Acta* 1187, 339145 (2021); doi: 10.1016/j.aca.2021.339145
- [6] N. Izadi, J. Strmiskova, M. Anton, J. Hausnerova, M. Bartosik, LAMP-based electrochemical platform for monitoring HPV genome integration at the mRNA level associated with higher risk of cervical cancer progression, *J Med Virol* 96, e70008; doi: 10.1002/jmv.70008
- [7] N. Pathak, J. Dodds, J. Zamora, K. Khan, Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis, *BMJ* 349, g5264 (2014); doi:10.1136/bmj.g5264