ELECTRODE BIOCHIPS COUPLED TO PERIODICAL AMPLIFICATION LAMP TECHNIQUE IN DIAGNOSTICS OF CERVICAL PRECANCER

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Introduction/Background Persistent infection with high-risk human papillomavirus (hrHPV) is a major etiological factor of cervical cancer. Hence, the effectivity of cytological screening can be improved by the implementation of hrHPV tests [1]. Current methods of HPV detection frequently involve expensive reagents and instrumentation or need for skilled personnel. Electrochemical methods of detection may address these challenges since they offer rapid detection times and require small, inexpensive instrumentation that is simple to operate.

Methodology We compared two different bioplatforms. Both utilized loop-mediated isothermal amplification (LAMP) to amplify HPV DNA from two most oncogenic HPV types, HPV16 and HPV18, taking 30-40 mins. Then, we used capture probes to bind amplified DNA, followed by an electrochemical detection using peroxidase reaction.

Results Using magnetic beads, we detected HPV DNA directly from crude lysates of cervical cancer cell lines (CaSkI, SiHa, HeLa) and from 19 clinical samples (patients with high-grade squamous intraepithelial lesion or healthy controls), without DNA extraction step [2]. Detection was possible from as little as 10 cells. We obtained excellent concordance of our assay with PCR, reaching 100% sensitivity for both genotypes, 81.82% specificity for HPV16 and 94.12% specificity for HPV18. Later, we omitted magnetic beads to detect HPV directly on gold electrodes, obtaining very good sensitivity and specificity when determining HPV16/HPV18 infection in 15 clinical samples when compared to the PCR [3].


PERFORMANCE OF A LAMP-BASED ELECTROCHEMICAL BIOASSAY FOR DETERMINATION OF HIGH-RISK HPV INFECTION IN CLINICAL SETTINGS

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Introduction/Background Persistent infection with the high-risk types of HPV is considered a crucial initiating factor in cervical carcinogenesis. Tests detecting the presence and especially the activity of HPV infection offer a new quality to screening and diagnostics. The limitation of these tests is, however, the price. Standardly used PCR tests are time consuming and instrument-intensive. A perspective alternative, the LAMP isothermal amplification coupled to an electrochemical detection, is presented.

Methodology We developed an assay for parallel detection of two most oncogenic high-risk HPV types, HPV16 and HPV18, by combining loop-mediated amplification (LAMP) of viral DNA, its separation using magnetic beads and detection with an electrochemical technique – amperometry – at carbon-based electrode chips.

Results Optimization of the method was first published on pilot files with a small number of cases. Later, we carried out a small clinical study using electrochemical LAMP-based assay for detection of HPV16/18 DNA in LBC samples obtained from 61 women undergoing conisation for cervical precancerous lesion.2 HPV16 and 18 assays were performed by LAMP isothermal amplification combined with electrochemical reading. The results were confirmed by PCR amplification with gel electrophoresis and two commercial HPV assays (Cobas and INNO-LiPA). The best concordance was obtained with the PCR, reaching very good specificity for both genotypes (>93%) and positive and negative predictive values over 90%.

Conclusion These data indicate that the EC-LAMP isothermal amplification may serve as an interesting alternative tool for rapid screening of oncogenic HPVs.

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REFERENCES

EVALUATION OF CERVICAL DYSPLASIA WITH NOVAPREP-MIR-CERVIX

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Introduction/Background Cervical cancer (CC) is one of the most common types of cancer and the fourth leading cause of cancer-related deaths in women. Cervical carcinogenesis is multistep process of the cervical dysplasia development and progression. Correct diagnostic and effective therapy of cervical dysplasia presents an important approach to reduce CC morbidity and mortality. MicroRNAs in cervical
Dermoscopy for genital lesions

Methodology

NOVAprep-miR-CERVIX is a new test-system based on RT-qPCR analysis of six miRNAs (miR-21-5p; miR-29b-3p; miR-145-5p; miR-451a-5p; miR-1246-5p and miR-1290-3p) in material of cervical smear. Test-system includes quality of material control and control of enzymatic reaction efficacy. Machine learning based of random forest algorithm was applied for RT-qPCR results evaluation. Cervical smear samples were obtained from 226 women: 114 samples of normal epithelium and 112 samples of cervical epithelium with high-grade intraepithelial lesion (HSIL) or carcinoma in situ (CIS) as a result of cytological evaluation. Moreover, any of HSIL/CIS diagnosis was confirmed histologically.

Results

The 38 samples from 216 (17.8%) did not pass quality controls and were excluded from analysis, NOVAprep-miR-CERVIX Index (miR-CERVIX-I varied from 0 to 1) was calculated on the basis of results of six miRNA analysis for remaining 178 samples. Difference in miR-CERVIX-I was statistically significant in two groups of samples formed on the basis of cytological/histological diagnosis (figure 1). Normal condition of cervical epithelium (miR-CERVIX-I < 0.49) was diagnosed with sensitivity 79.2%, specificity 80.46%. HSIL was diagnosed with sensitivity 70.83%, specificity 97.22% (miR-CERVIX-I > 0.78). Moreover, intermediate value of miR-CERVIX-I (between 0.5 and 0.77) is supposed to reflect condition of low-grade intraepithelial dysplasia.

Conclusion

NOVAprep-miR-CERVIX can be applied for cervical dysplasia diagnostic and management as a test system complimentary to standard methods.