

## **Detection of multiple HPV types in liquid biopsies of cervical neoplasia**

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### **Background & objectives**

Over 95% of cervical cancers and their precancerous lesions are caused by human papillomavirus (HPV). Cell-free (cf) HPV DNA detection in blood samples may serve as a marker for cervical cancer.

### **Methods**

In our study, an HPV panel detecting 24 types simultaneously was developed for liquid biopsy approaches and tested on HPV-positive cell lines, plasmid controls and cervical HSIL-positive smear samples (n=52). Validation was done in cfDNA blood samples (n=40) of cervical cancer patients.

### **Results**

This mass spectrometry-based HPV assay showed proficient results in cell lines and viral plasmids with a sensitivity of 1 IU/ $\mu$ L for HPV16/18 and 10 GE/ $\mu$ L for HPV11/31/33/39/45/51/52/58/59 as well as a specificity of 100% for these tested HPV types. In cervical smear samples, HPV DNA was detected with a sensitivity of 98.14% and a positive predictive value of 0.98 (49/50). The overall agreement between the HPV panel and clinical records was 97.2% ( $\kappa=0.84$ ). In cervical cancer cfDNA, 26/40 (65.0%) were tested positive for any HPV type. The distribution of HPV types showed most infections are due to hrHPV (24/26 plasma HPV positive). HPV-positive samples were found in all FIGO stages, with the highest positivity ratio in FIGO III and IV (87.5% combined). Still, 12/23 (52.2%) of FIGO I patients had a positive HPV plasma status.

## **Conclusion**

The assay showed reliable results for detecting a large number of HPV types in a multiplex mass spectrometry-based assay in cervical smear as well as in cfDNA.