HPV DNA DETECTION ASSAYS FOR CERVICAL SCREENING

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Summary
Various high-risk HPV (HR HPV) DNA assays have been developed that allow detection of a broad spectrum of HR HPVs. Two of these assays [Hybrid Capture 2 (hc2) and GP5+/6+-PCR] have shown in large clinical trials a superior clinical sensitivity for cervical (pre)cancer compared to cytology and an optimal balance between clinical sensitivity and specificity. Comparative studies showed that an increased sensitivity for HR HPV relative to GP5+/6+-PCR and/or hc2 results in a dramatic decrease in clinical specificity, whereas on the other hand a decreased sensitivity for virus leads to a decrease in sensitivity for (pre)cancer. These data argue for guidelines on HR HPV test requirements for cervical screening purposes.

Key words: human papillomavirus, cervical cancer, high-grade CIN, HPV DNA testing, clinical performance

The fact that high-risk human papillomavirus (HR HPV) infection is a necessary cause of cervical cancer offers possibilities to implement HR HPV testing in cervical cancer screening to improve the efficacy of the screening program. Many test systems have been developed that can detect the broad spectrum of HR HPV types in one assay. An extensive overview of available HPV detection methods has recently been described (1). Most of these HR HPV assays comprise DNA detection methods based on either target amplification utilizing PCR or signal amplification.

As a result of the heterogeneity between HR HPV types, the majority of DNA PCR methods make use of consensus primers that target the same conserved region within the viral genome, though also multiplex systems targeting different regions for different types in one reaction have been described. The most commonly applied consensus PCR assays include GP5+/6+-PCR (a modified version of GP5/6), PGMY09/11 (a modified version of MY09/11), and SPF10 (1). Detection of a PCR product can easily be performed by enzyme-immuno-assays (EIA) that use a cocktail of type-specific or a mix of universal probes. Genotyping on the generated PCR products is mostly carried out by reverse hybridization formats using type-specific oligonucleotide probes immobilized to filters/strips (like reverse line blot (RLB), line probe assay (LiPA), linear array), micro-arrays (micro-chip based oligonucleotide arrays), or microsphere beads (bead-based multiplex HPV genotyping). According to our experience, the various reverse hybridization on consensus GP5+/6+-PCR products perform equally well and reveal highly concordant typing results. Hence, the clinical value of these typing assays largely depends on the clinical performance of the PCR system generating the products that are used for typing.

Other HPV DNA detection assays are based on signal amplification and either have a liquid-phase or an *in situ* hybridization (ISH) format. The commercially available and FDA (USA) approved hybrid capture 2 (hc2, Qiagen, Gaithersburg, MD., USA) is an example of a liquid-phase format and detects 13 genital HR HPV types using a mixture of full-length RNA probes. Unlike PCR formats, the signal amplification formats do not enable HPV genotyping in one assay run.

With respect to all above mentioned assays, it should be realized that for screening and clinical practice purposes the detection of HR HPV is not inherently useful unless it is found in the context of cervical precancer (high-grade cervical intraepithelial neoplasia – CIN) or cancer. With regard to this aspect, there is sufficient evidence that the viral load (ie. the amount of viral DNA in a sample) is an important variable since very low viral loads reflect clinically irrelevant, mostly transient, HR HPV infections. As such, detection of such low copy numbers of viral DNA will have a negative impact on the clinical specificity for detection of high-grade CIN and cervical cancer. In fact, for screening purposes, HR HPV DNA tests should reach an optimal balance between clinical sensitivity and specificity. Current clinically

validated tests displaying such balance, for example hc2 and GP5+/6+-PCR have repeatedly demonstrated both clinical sensitivity and clinical specificity of about 90–95% for the detection of high-grade CIN and cancer (2-5). One should be cautious against misguided attempts to increase the sensitivity of HPV assays, as possible gains in clinical sensitivity will be trivial while at the same time the adverse consequence will be a dramatic increase in the number of false positives (i.e. increased detection of HR HPV positive women without high-grade CIN or cervical cancer). For example, comparison of the clinical performance of an ultra-sensitive PCR assay to the HR HPV GP5+/6+-PCR in women over 30 years of age revealed that the ultra-sensitive assay did not lead to an increase in clinical sensitivity for high-grade CIN or cervical cancer, but instead to a significantly decrease in clinical specificity as compared to that of the GP5+/6+-PCR (6). The extra positivity scored by the ultra-sensitive assay mainly involved infections characterized by a very low viral load that did not result in high-grade CIN or cervical cancer. Conversely, hc2 and GP5+/6+-PCR assays are compatible in terms of clinical performance (7). Moreover, viral load analysis appeared not useful to improve the clinical specificity of the GP5+/6+-PCR while it resulted in a marked reduction of clinical sensitivity for high-grade CIN or cervical cancer.

Taken together, for application in cervical screening a candidate HR HPV test should have similar clinical characteristics as the hc2 or GP5+/6+-PCR tests. Guidelines for requirements of HR HPV DNA tests to be used in primary cervical cancer screening need to be formulated to reduce both false positives and false negatives in term of high-grade CIN and cervical cancer.

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