

A new multiparameter assay to assess HPV 16/18, viral load and physical status together with gain of telomerase genes in HPV-related cancers

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Oncogenic human papillomavirus (HPV) is the most important risk factor for cancer of the uterine cervix and a subgroup of head and neck cancers. Viral load has been associated with persistence of infection, whereas integration of HPV into the host cell genome is associated with transition to invasive disease. Viral integration is frequently correlated with loss of viral E2 and gain of the telomerase-related genes *TERC* and *TERT*. The objective of this study was to develop a rapid and sensitive multiplex ligation-dependent probe amplification (MLPA) assay for the simultaneous analysis of viral load, integration and copy number gain of *TERC* and *TERT* in HPV16/18-associated lesions. The performance of the assay was tested for HPV vs. human gene copy number ratios ranging from 0.1 to 100 and for percentages of integration ranging from 0 to 100%. The model systems used include plasmid mixtures and the HPV-positive cell lines SiHa, HeLa and CaSki described to contain a range of 2–600 viral copies per cell. In samples with low-viral load, viral integration can be reliably determined when more than 30% of the virus is integrated. Gain of the telomerase-related genes in the cell lines as determined by our MLPA assay was in accordance with data reported in the literature. Our study demonstrates that within a single MLPA-reaction viral type, load, integration and gain of *TERC* and *TERT* can be reliably determined, which will improve risk assessment for patients suspected for HPV infection.

Human papillomavirus (HPV) plays a causal role in the development of several cancer types¹ and has been identified in the majority of uterine cervical and anal cancer cases^{2,3} and in approximately half of the tonsil and penile cancers.^{4,5}

Key words: uterine cervical cancer, head and neck cancer, viral integration, *TERC*, *TERT*

Additional Supporting Information may be found in the online version of this article

Abbreviations: HPV: human papillomavirus; MLPA: multiplex ligation-dependent probe amplification; *TERC*: Telomerase RNA Component; *TERT*: Telomerase Reverse Transcriptase; FISH: fluorescence *in situ* hybridization; CGH: comparative genomic hybridization; *MSH2*: MutS homolog 2; bp: basepairs; FAM: carboxyfluorescein; CNR: copy number ratio; qPCR: quantitative PCR

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Most HPV-related cervical intraepithelial precursor lesions are known to regress spontaneously, which implicates that although HPV is a necessary cause, it is not independently causative for cancer. Additional factors play a role in the progression and/or prognosis of these (pre)malignant lesions, including, e.g., individual susceptibility, integration of the virus and genomic instability, as described later.

In the past years, many studies have focused on the identification of markers that can predict the progression of cervical intraepithelial neoplasia to cervical cancer, which may thus help to discriminate regressive from progressive HPV-related lesions. The markers include HPV type,^{4,6} viral load^{7,8} and physical status^{9,10} and gain of telomerase-related genes.^{11–14} HPV16 and 18 are the most prevalent oncogenic types in cervical cancer and are found to be responsible for more than 70% of cervical carcinomas.^{15,16} In tonsillar squamous cell carcinomas, HPV16 is predominantly found in HPV-positive cases.⁴ Furthermore, in cervical lesions a high HPV16 viral load is more likely to lead to a persistent infection than a low-viral load,^{7,17,18} which in turn enhances the likelihood of progression to cancer.

It has been found that part of the HPV genome will be lost on integration into the host genome. This predominantly concerns the *E2* gene but may also include the *E1* or *L1* gene.^{3,19,20} Some studies have demonstrated that there is a very wide range of integration-related disruption sites in the viral genome.^{20–22} Most of these deletions will cause silencing of the *E2* gene, which in turn will lead to a