



# Detection and Genotyping of HPV Infection Using a New Method Based on Real-Time PCR

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## Abstract

The causalGiven the known relationship between the HPV infection and some malignancies, it is critical to develop methods for quick detection and quantitation of certain HPV types while encountering a suspected lesion. Early HPV detection is greatly important in monitoring and treating the disease development and progression. Detection of the viral DNA using PCR is the standard, noninvasive method for detecting cervical HPV infection. In the present study, we intended to develop a TaqMan genotyping assay that targets two types of high-risk HPV types (HPV 16 & 18) and two of the low-risk types (6 & 11).

The study included 75 samples positive for HPV, of which 37 were positive for HPV types 16 and 18, while 38 were positive for HPV types 6 and 11. The samples had been confirmed by a reference kit before. The samples underwent real-time PCR. Each reaction consisted of the 1X CAPITAL™ qPCR Probe Master Mix, specific primer pairs for HPV, and fluorescent-tagged probes.

According to our findings, all the samples genotyped using this method were compatible with the results by the reference kit, which was remarkable.

In conclusion, our type-specific approach based on real-time PCR could detect the entire samples positive for four types of HPV.

## INTRODUCTION

Papillomaviridae is a diverse family of non-enveloped, small circular viruses containing double-stranded DNA. These viruses can infect different mammals and birds (1). It has been shown that they cause pathologies ranging from benign warts to malignant lesions in humans. HPV is proved to be the cause of more than 90% of all cervical cancer cases (2), the third most common cancer in women globally. However, as a result of cytology-based screening, it is only the 15<sup>th</sup> most common cause of cancer-related death in women in Western Europe. HPVs are divided into two groups of low-risk and high-risk based on their association with malignancy (3). HPV types 6 and 11 are the most commonly detected types of low-risk HPV in genital and anal warts, representing 90% of these cases (4), while oncogenic HPV types 16 and 18 account for 70% of the HPV-related cervical cancers. It has been shown that HPV viral loads are positively correlated with disease progression in cervical cancer (5). Similarly, elevated HPV viral loads have been detected in HPV-16-associated oropharyngeal squamous

cell carcinomas (6). These associations highlight the importance of detection, differentiation, and quantitation of low-risk and oncogenic HPV infections to monitor and treat disease development and progression (7-8). Detection of the viral DNA using PCR is the standard, noninvasive method for cervical HPV infection detection. PCR is a sensitive method for detecting HPV DNA. Current real-time PCR assays for HPV detection, such as the RealTime High Risk (HR) HPV assay (Abbott) and the Cobas®4800 HPV Test (Roche Molecular Diagnostics), are commercially available. However, none of them are capable of detecting and typing all high-risk HPV types in a clinical high-throughput setting (9). Therefore, the present study intended to develop a TaqMan genotyping assay that targets two types of high-risk HPV types (HPV 16 & 18) and two of the low-risk types (6 & 11).

## METHODS AND MATERIALS

75 HPV positive samples were used in this study, which The study included 75 samples positive for HPV, of which 37 were positive for HPV types 16

and 18, while 38 were positive for HPV types 6 and 11. The samples had been confirmed before the study using the reference kit REALQUALITY RQ-Multi HPV Detection (AB ANALITICA s.r.l, Italy). First, DNA was extracted from liquid samples using the One-4-All Genomic DNA Miniprep Kit (BioBasic, Canada). Amplification was performed in a single real-time PCR reaction for 6/11 and 16/18 types, while beta-globin was used as the control. The primers and probes used are listed in Table 1. Real-time PCR reactions were performed using the 4X CAPITAL™ qPCR Probe Master Mix (biotechrabbit

GmbH, Germany). Each reaction consisted of the 1X CAPITAL™ qPCR Probe Master Mix, specific primer pairs for HPV, and fluorescent-tagged probes for HPV types 6, 11, 16, and 18 in a total reaction volume of 20 µL. Probes for HPV types 16 and 18 were labeled with fam color, those for HPV types 6 and 11 were labeled with hex color, and beta-globin controls were labeled with Rox color. The human beta-globin gene was detected at a single copy in normal cells. Reactions were performed using a DT-prime (DNA-technology, Russia). Primer and probe sequences.

Oligonucleotide	Sequence Sequence
HPV6LR	F: 5'-TGGGGTAATCAACTGTTTGTACTGTGGTA-3' R:5'-GCATGTACTCTTTATAATCAGAATTGGTGTATGTG-3' hex-GACATTATGTGCATCCGTAAC-TAC-BHQ
HPV11LR	F: 5'- CTGGGGAACCACCTTGTGTACTGTG-3' R:5'- CGCATGTATTCCTTATAATCTGAATTAGTGTATGTA-3' hex-GACACTATGTGCATCTGTGTCTAA-BHQ
HPV16HR	5'-TTGTTGGGGTAACCAACTATTTGTTACTGTT-3' 5'-CCTCCCCATGTCTGAGGTACTCCTTAAAG-3' FAM-GTCATTATGTGCTGCCATATCTACTTC-BHQ
HPV18HR	5'-GCATAATCAATTATTTGTTACTGTGGTAGATACCACT-3' 5'-GCTATACTGCTTAAATTTGGTAGCATCATATTGC-3' HEX-AACAATATGTGCTTCTACACAGTCTCCTGT-BHQ
β-globin	5'-CAGGTACGGCTGTCATCACTTAGA-3' 5'-CATGGTGTCTGTTTGAGGTTGCTA-3' ROX-GCCCTGACTTTTATGCCAGCCCTG-BHQ

## RESULTS

DNA isolated from 75 samples from women who were HPV-positive underwent real time PCR, including 37 samples positive for HPV types 16 and 18 and 38 samples positive for HPV types 6 and 11. According to our findings, all the samples genotyped by this method were compatible with the results by the reference kit, which was remarkable.

## DISCUSSION

The causalGiven the known relationship between the HPV infection and some malignancies, it is critical to develop methods for quick detection and quantitation of certain HPV types while encountering a suspected lesion (10). Early detection lets us assess the precancerous lesions and helps in decision-making for either treating the lesions before progression to a more severe form of disease or allowing it to resolve on its own (11). Moreover, low-risk types of HPV have negative impacts on the quality of life, indicating the importance of tracking and identifying HPV types involved in the development of warts (12). The development of assays capable of distinguishing between the common types of high-risk and low-risk HPVs involved in malignancies and warts can facilitate understanding of HPV

epidemiology and pathology (13). PCR has several features that make it an attractive approach for HPV detection compared to alternative approaches: primers specified for the viral oncogenes, a small amplicon size, HPV genotyping, HPV viral load assessment, high-throughput application, and clinical validation (14). In the present study, we intended to develop a TaqMan genotyping assay that targets two types of high-risk HPV types (HPV 16 & 18) and two of the low-risk types (6 & 11). The study included 75 samples positive for HPV, of which 37 were positive for HPV types 16 and 18, while 38 were positive for HPV types 6 and 11. The samples had been confirmed using a reference kit before. According to our findings, all the samples genotyped by this method were compatible with the results by the reference kit, which was remarkable. The value of HPV type identification in clinical routine is still debated (15). However, specific detection of HPV 16 and 18 may be important because they imply a higher risk for progression of dysplasia. Moreover, typing has a clinical value in follow-up assessment interpretations after an identified HPV infection because it may allow distinction between the persistent infection and re-infection with new types (16). In conclusion, our type-specific approach based

on real-time PCR could detect the entire samples positive for four types of HPV. Finally, it should be pointed out that our method in its present form has the limitation of genotyping only 4 HPV types in each reaction. Therefore, we are currently evaluating a multiplex format.

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