

Applying Multiplex Real-time PCR to Evaluate E6/E7 Expression in Human Papillomavirus

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BACKGROUND

In HPV genotype detection testing, to definitively determine whether the detected HPV genotype is actually invading and proliferating within epithelial cells, it is essential to detect its E6 and/or E7 mRNA^[1,2]. Therefore, this is a test currently recommended to be included with HPV genotype detection testing, and we have developed and perfected this test to complement the HPV genotype detection test that we have been implementing

AIMS OF THE STUDY

This study aimed to determine the detection rate of E6 and E7 mRNA from cervical smear samples that had HPV genotype detection results and collected PAP smear samples that detected epithelial cell abnormalities, in order to assess the detection rate of cases where HPV genotypes actually invaded and proliferated in epithelial cells.

MATERIALS AND METHODS

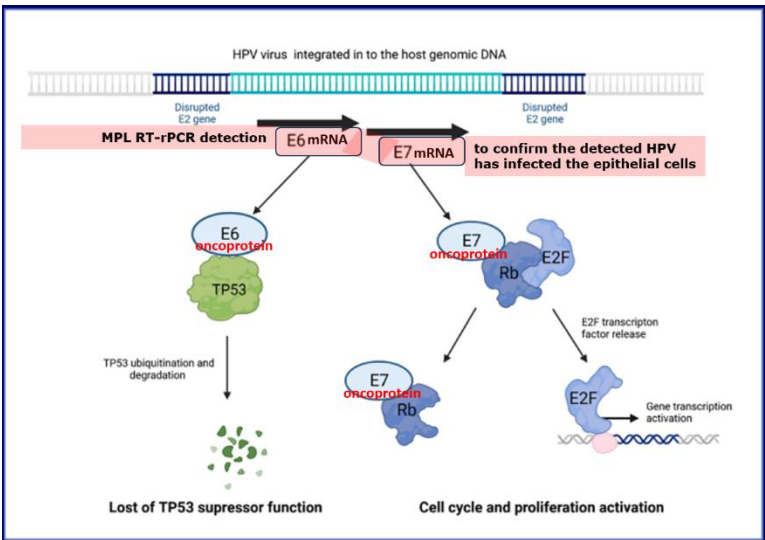
Retrospective analysis was performed on 587 cervical cell samples at the Vietnam Institute for Clinical Microbiology Research and Development (VCM). Total RNA was extracted and processed with RNase-free DNase to ensure DNA removal. We used the reverse transcriptase multiplex real-time PCR (RT MLP-rPCR) targeting the detection of E6/E7 mRNA of 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and 2 low-risk types (6, 11).

RESULTS

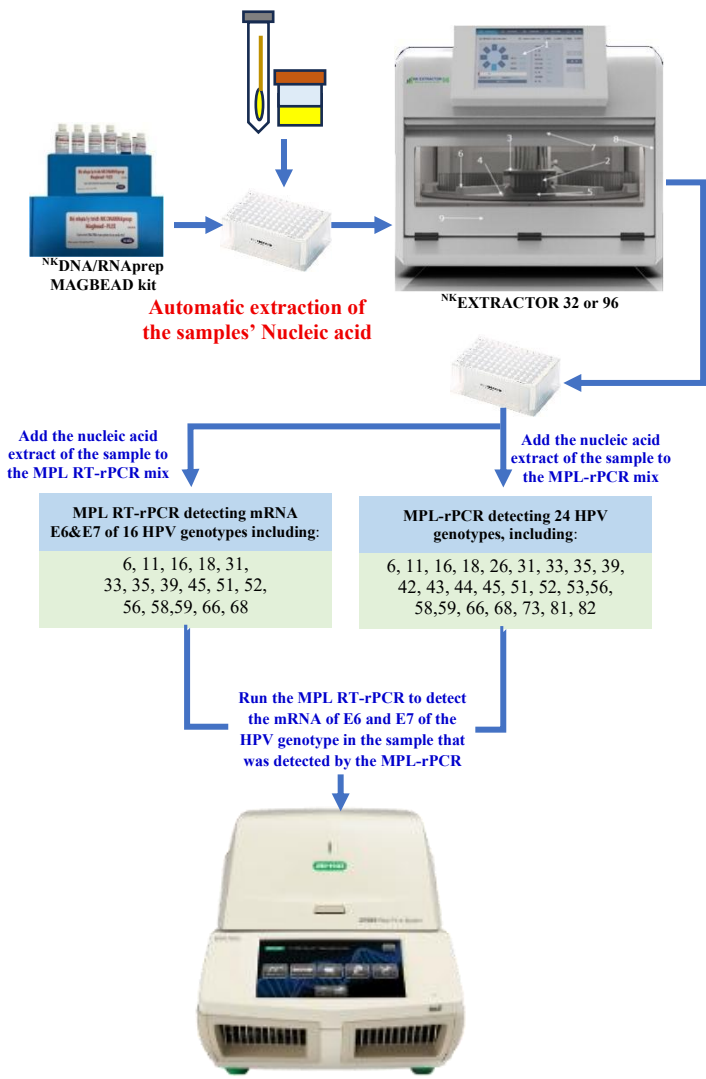
The overall mRNA positivity rate was 39.2% (230/587), including 27.1% with simultaneous detection of E6 and E7, 6.6% with only E7, and 5.1% with only E6; 60.8% were negative for E6/E7 mRNA. The dominant HPV genotypes in the E6/E7 mRNA-positive samples were HPV52 (22.2%), HPV58 (20.9%), HPV16 (14.8%), HPV39 (11.3%), and HPV51 (10.4%). HPV58 showed polymorphism, while HPV66/68 showed no E6/E7 mRNA signal despite the presence of DNA, indicating that the two genotypes did not invade epithelial cells in the study cases. Additionally, the study analyzed similarities and differences when compared with PAP smear results.

DISCUSSIONS AND CONCLUSION

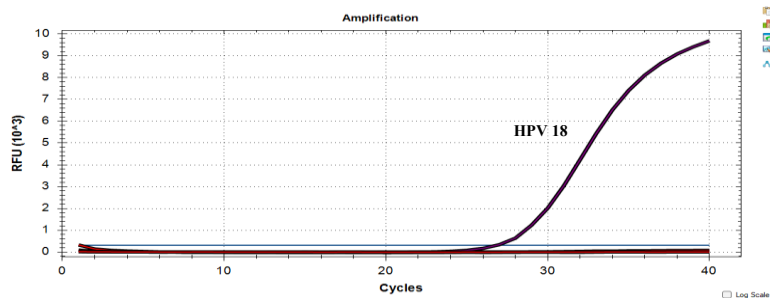
Research results show that E6/E7 mRNA detection testing is essential when combined with HPV genotype detection testing to determine whether the pathogen has truly invaded the genital epithelial cells of patients. Therefore, in many countries, HPV E6/E7 mRNA detection testing is now recommended as a replacement for PAP smear testing, as PAP smear results are not truly specific for this purpose. However, because commercial E6/E7 mRNA testing products are still quite expensive, we hope that after this study and many future studies, this test will be more easily implemented due to its affordable cost.



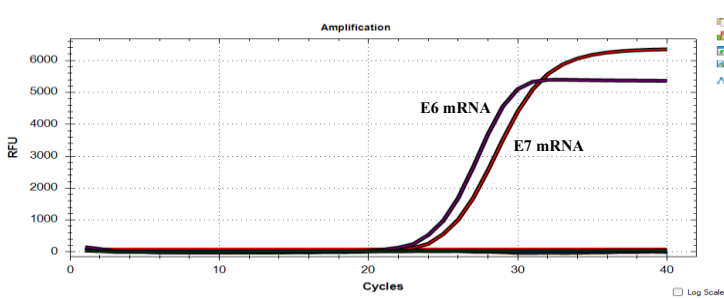
Picture 1: After infected into the epithelial or endothelial cells, the DNA of HPV will integrate into the host genomic DNA and the E6 and E7 mRNA of the HPV will be cloned to synthesis the E6 and E7 oncoprotein. The E6 oncoprotein will act to lost the TP53 function, and the E7 oncoprotein will activate the HPV infected cell cycle and the uncontrol proliferation activation.



Picture 2: The methods in the MPL-rPCR to detect the 24 HPV genotypes and in the MPL RT-rPCR to detect E6&E7 mRNA of 16 HPV genotype. These methods include: (i) Nucleic acid extraction from the samples; (ii) Run the MPL-rPCR to detect the existing of the HPV genotype in the sample; (iii) Run the MPL RT-rPCR to detect the mRNA of E6 and E7 of the HPV genotype in the sample that was detected by the previous MPL-rPCR



Picture 3: One of the results of the MPL-rPCR of the tested sample showed that the HPV genotype 18 was detected



Picture 4: From that sample (in picture 3), the MPL RT-rPCR detected the presence of the E6 mRNA and E7 mRNA of the HPV genotype 18

KEY WORDS

HPV genotypes, E6/E7 mRNA; Reverse transcriptase multiplex real-time PCR (RT MLP-rPCR).

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