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Real-Time Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Human Papillomavirus 16 in Oral Squamous Cell Carcinoma

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Abstract : Human papillomavirus (HPV) is an important risk factor for development of oral cancer. HPV16 is the most common type found in HPV-positive squamous cell carcinoma. In the present study, we established a real-time loop-mediated isothermal amplification (real-time LAMP) for detection of HPV16. A set of six primers was specially designed to recognize eight distinct sequences of HPV16-E6. Detection and quantification was achieved by real-time monitoring using a real-time turbidimeter based on threshold time required for turbidity in the LAMP reaction. LAMP reagents (MgSO4, dNTPs, Bst polymerase concentrations) and various incubation times and temperatures were optimized. The sensitivity was determined using 10-fold serial dilutions of HPV16 standard strain. The specificity of was evaluated using other HPV genotypes. The optimized method was established with specifically designed primers by real-time detection in approximately 30 min at 65°C. The limit of detection of HPV16 using the LAMP assay was 10 pg/ml that could be detected in 30 min. The LAMP assay was 10 times more sensitive than the conventional PCR in detecting HPV16. No cross-reactivity with other HPV genotypes was observed. This quantitative real-time LAMP assay may improve diagnostic potential for the detection and quantification of HPV16 in clinical samples and epidemiological studies due to its rapidity, simplicity, high sensitivity and specificity. This assay will be further evaluated with HPV DNAs of saliva from patients with oral squamous cell carcinoma. Acknowledgement: This study was financially supported by the ScienceFund Grant, Ministry of Science, Technology and Innovation (305/PPSG/6113219).

Keywords : Oral Squamous Cell Carcinoma (OSCC), Human Papillomavirus 16 (HPV16), Loop-Mediated Isothermal Amplification (LAMP), rapid detection
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