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The Cancer Control Challenge:
Better Understanding It to Better Face It

RAPID DIAGNOSIS OF HUMAN PAPILLOMAVIRUS (HPV) USING PCR-LAMP: A POINT-OF-CARE APPROACH

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Introduction: Cancer is a Non-Communicable Chronic Disease (NCD) characterized by a group of multifactorial diseases, including viral infections. Human Papillomaviruses (HPV) are associated with the occurrence of malignant neoplasms as well as other health conditions, such as cutaneous and genital warts. HPV belongs to a large family of non-enveloped, double-stranded DNA viruses, with over 220 identified types, and is involved in various carcinogenesis processes, including head and neck cancer (HNC), cervical, anal, vulvar, and penile cancers. Loop-mediated isothermal amplification (PCR-LAMP) requires minimal instrumentation to synthesize DNA/RNA in vitro. The technique consists of two phases—cyclic and non-cyclic—and requires the design of specific primers: BIP (Backward Inner Primer), FIP (Forward Inner Primer), B3P (Backward 3 Primer) and F3P (Forward 3 Primer). LAMP emerges as an alternative for molecular diagnostics, enabling testing in resource-limited settings in a shorter time compared to conventional PCR, allowing sample collection and diagnosis without the need for large laboratory facilities, characterizing a Point-of-Care approach. **Objectives:** To develop a loop-mediated isothermal amplification (PCR-LAMP) technique for the rapid diagnosis of HPV. **Methods:** Biological samples from HNC patients were collected through oral swabs, with smears taken from multiple points in the oral cavity and subsequently stored at -80ºC. Viral DNA extraction followed a protocol using PBS (Phosphate-Buffered Saline) and Proteinase K. The quantification of viral DNA samples was performed using the Qubit 4.0 platform. Loop-mediated isothermal amplification (LAMP) was carried out using the Bst2.0 DNA Polymerase kit (Cellco). Target sequences were selected from the NCBI (National Center for Biotechnology Information) database, aligned, and stable regions were identified. The LAMP primers were designed using UGENE 49.1 and LAMP Primer Explorer V.4 software. PCR-LAMP reactions were performed in a thermocycler at 60ºC for 60 minutes and 80ºC for 10 minutes. Results were interpreted through electrophoresis, with fluorescence visualization used to determine the method's sensitivity. **Results:** Initial amplification verification was observed through electrophoresis, where standardization primers (β-Actin) displayed bands corresponding to the expected molecular weight, as

did the HPV primers. Rapid diagnostic visualization was successfully achieved by adding the SYBR Safe 10X intercalating dye—which binds to double-stranded DNA—to the PCR-LAMP product, followed by UV light exposure for detection. Positive samples and negative controls were distinguished by the intensity of emitted fluorescence, which varied according to the concentration of viral DNA present, with positive results obtained at 10 ng/mL of DNA. **Conclusion:** The PCR-LAMP technique demonstrated fidelity and specificity to HPV, regardless of type, providing easily visualized results and optimizing sample collection and processing time. This method is accessible for large-scale screening programs due to its low operational cost, embodying the key features of a Point-of-Care approach.

Keywords: PCR-LAMP; Human Papillomavirus; Point-of-Care Diagnosis.