Cleveland Clinic Innovations

## PRODUCT

Single-Tube hrHPV Genotype-Specific Real-Time Polymerase Chain Reaction (qPCR) Assay for Detection of Cell-Free hrHPV DNA in human plasma and body fluids

## INDICATIONS

Human Papillomavirus Virus (HPV), HPV-related neoplasms

## VALUE PROPOSITION

- Highly sensitive and specific diagnostic assay
- Reagents optimized for hrHPV detection in human plasma and body fluids
- Primers and probes capable of broader HPV16 genomic coverage compared to current commercial methods
- Does not rely on sophisticated or expensive equipment, or resource intensive procedures / reagents

## DEVELOPMENT STAGE

Diagnostic platform available for partnering and licensing

## INTELLECTUAL PROPERTY

Patent Pending

## PUBLICATION

Clark, P. *et al*. Highly Multiplex Detection and Quantitation of Plasma Cell-Free Human Papillomavirus-16 DNA in Oropharyngeal Carcinoma. *International Journal of Radiation Oncology*, Volume 120, Issue 2, e35.

## CONTACT INFORMATION

Saqib Sachani, PhD, MBA
Director, Business Development and Licensing
sachans@ccf.org
216.672.1913

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# Diagnostic Method for Multiplex Detection and Quantification of Cell-Free HPV-16 DNA

*Jacob Miller MD, Payton Clark BS, Shlomo Koyfman MD, Timothy Chan MD, PhD – Cleveland Clinic Lerner Research Institute*

## UNMET NEED

Oropharyngeal squamous cell carcinoma (OPSCC) is the most common type of HPV-associated cancer in the United States. This head and neck squamous cell carcinoma has increased in incidence despite steady high-risk human papilloma virus (hrHPV) vaccination in adolescents and young adults. This increasing incidence has been attributed to the long latency of hrHPV and absence of population-level screening. Latently-infected adults will develop HPV-associated neoplasms and suffer from the associated cancer-related morbidity and mortality. .

At present, there is a severe unmet need in hrHPV-specific diagnostic technologies that enable the effective detection and longitudinal monitoring of cell-free hrHPV among patients with HPV-associated neoplasms. Due to the time-sensitive / anatomic stage driven treatment of HPV-related neoplasms, effective diagnostics are paramount to ensuring effective malignancy surveillance, minimal residual disease detection,and biomarker-adapted therapy. Presence of hrHPV cell-free DNA and particularly HPV16 cell-free DNA has been identified as a biomarker for relapse of OPSCC. However, currently marketed products either lack sensitivity for minimal residual disease detection or rely upon complex and costly technologies that are not widely-available. This is particularly relevant for patients undergoing serial testing during treatment and years of follow-up.

## SOLUTION

Researchers at Cleveland Clinic have developed a highly-multiplex single-tube qPCR assay for detection of cell-free hrHPV DNA in human plasma and body fluids. The primers and probes used in the assay are specifically optimized for detection of short fragments of cell-free DNA with greater HPV16 genomic coverage while still achieving highly sensitive and specific detection for the HPV16 genotype of interest. This assay format offers substantial improvements over currently available methods as it offers an effective, low-cost solution to HPV detection, and does not require sophisticated equipment or reagents. The analytical performance of this technology, combined with its ease of use enables healthcare providers to intervene earlier in cases of hrHPV-related malignancies, leading to improved clinical outcomes in these patients.

**Advantages:**

1. **Low cost and complexity:** This one-step single-tube qPCR format uses standard techniques and instrumentation available in clinical pathology laboratories conducting routine NAAT. This assay does not rely on digital PCR which are unavailable in most clinical laboratories. In contrast to hybrid-capture HPV NGS with ~48 hour turnaround time, this assay can be performed in a single step after nucleic acid extraction with rapid turnaround time and does not require a multi-step library preparation. Moreover, the assay is suitable for automated extraction/amplification platforms and longitudinal monitoring.
2. **Optimization for cell-free DNA and genomic coverage:** The assay is designed to detect short fragments of cell-free DNA in plasma/body fluids, in contrast to diagnostics designed for cervical cytology specimens. Furthermore, analytical sensitivity of the assay is improved by increasing HPV16 genomic coverage from ~1-2% with HPV16 E6/E7 qPCR/dPCR to ~16% while avoiding primer dimerization and cross-genotype nonspecific amplification.