Quantitative detection of cell free HPV DNA in liquid biopsy samples for cancer monitoring

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1. Abstract

Around 690.000 cancer cases are attributable to human papillomavirus (HPV) infection globally every year, most of them cervical (570.000) and oropharyngeal (42.000) cancers. Based on our current lab developed test*, that can quantitatively detect cell free HPV16/18 DNA in plasma samples, we strive to develop an assay in a Kit format with matched performance that we will distribute directly to customers for use in sample preparation.

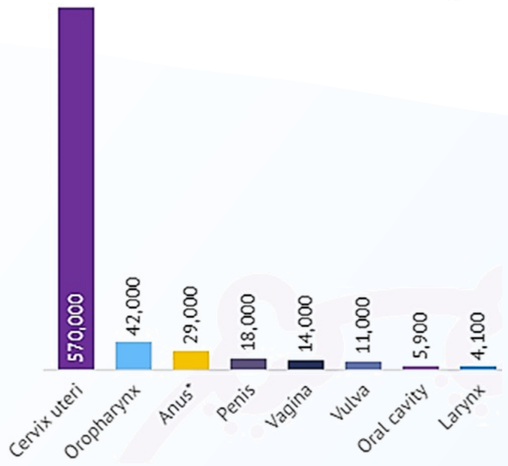
We use PCR for targeting endogenous cell-free HPV 16/18 DNA isolated from plasma. In conjunction with an internal quantification method, this library is sequenced on a Nanopore Sequencing device to identify HPV16/18 DNA in a range of 5-50,000 copies.

Utilizing the Nanopore Sequencing technology, a low investment sequencing platform with real time analysis capability, the customer can batch up to 32 samples or run individual samples at almost equal cost.

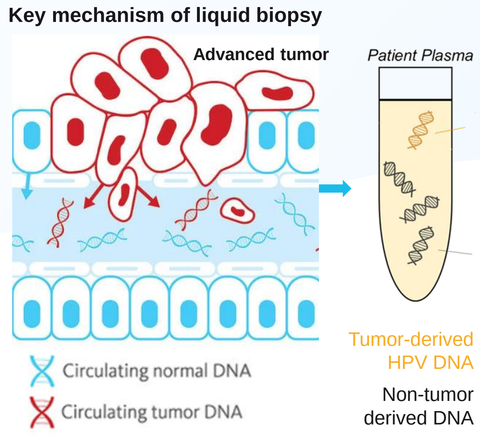
Among other applications this method can be used to monitor tumor burden of HPV associated cancers. This kit is designed for quick turn-around time, ease of use and low sequencing cost.

**HPV-SEQ, running in our CLIA accredited Lab at Sysmex Inostics Inc., Baltimore, MA, USA[1])*

Cancer cases associated with HPV infection (global)



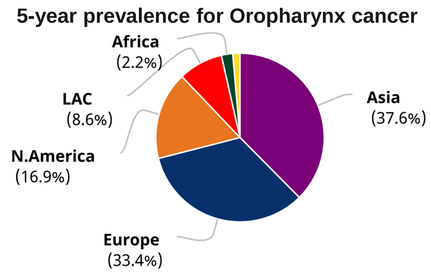
Key mechanism of liquid biopsy



2. Background

HPV infection has been shown as a driver of oropharyngeal cancer which is a type of head and neck cancer. HPVs cause around 70% of oropharyngeal cancer in the United States but are also widely distributed in other areas of the world. HPV associated oropharyngeal cancer parallels with better prognosis compared to those without[2]. Together with other biomarkers, HPV from cfDNA in plasma seems to be a promising marker for predicting and monitoring treatment responses in head and neck cancer patients and refining patient treatment strategies[3].

5-year prevalence for Oropharynx cancer



- Liquid biopsy is a non-invasive alternative to surgical biopsies
- Cell-free HPV DNA (cfHPV-DNA) represents a promising surrogate for disease burden in patients with HPV-associated cancers, e.g., HNSCC
- Dynamic changes in HPV DNA levels have been shown to correlate with treatment response
- Longitudinal HPV DNA monitoring may demonstrate utility for surveillance of cancer recurrence after curative-intent therapy
- CLIA-validated HPV-SEQ assay shows robust quantitative detection of HPV 16/18 cfDNA across a broad dynamic range over five orders of magnitude[1]

3. Aim

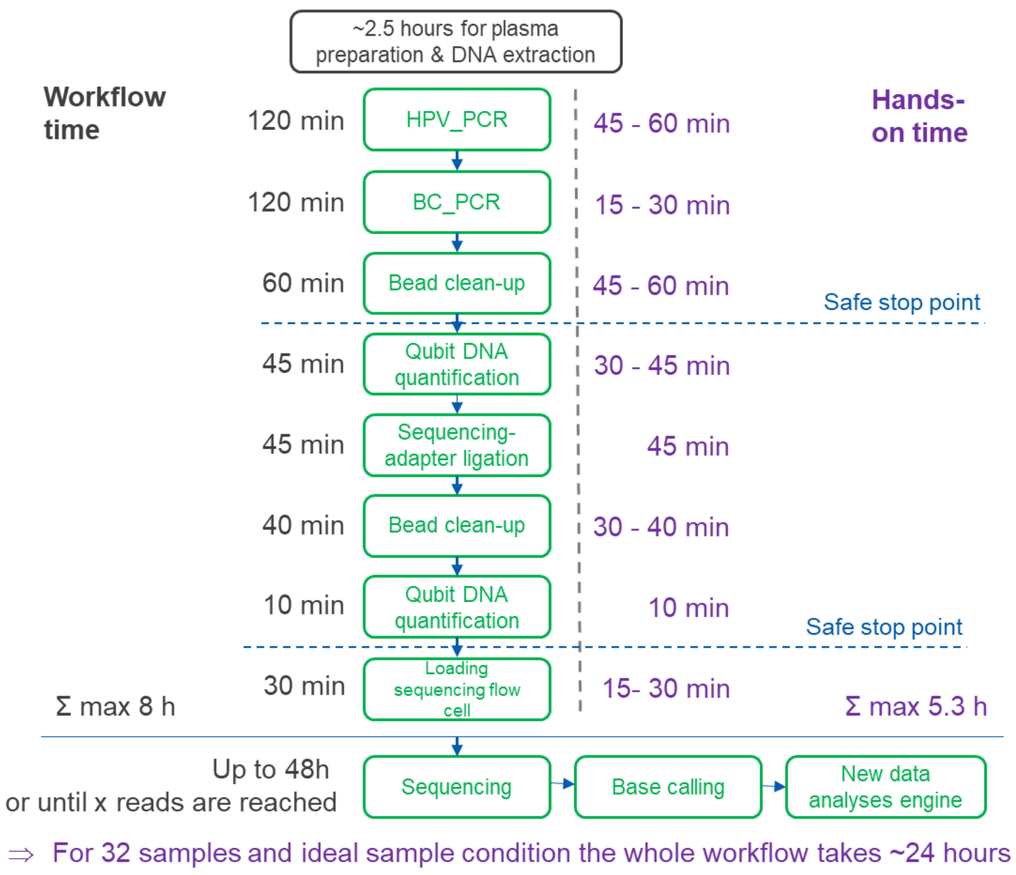
The assay is aimed to detect cell-free HPV 16/18 DNA isolated from blood plasma samples and quantifies its abundance in a range of 5-50,000 copies. Since this product is for RUO, it had to be cost efficient and accommodate single sample testing or larger batch processing. For upscaling the sample throughput, the workflow had to be adaptable to fully integrated automation. Feedback of key opinion leaders included a strong interest in a de-centralized approach of sequencing based quantitative HPV detection, especially in locations not properly equipped for such purpose at present.

4. Workflow

The workflow starts with blood collection and extraction of the cell-free DNA (cfDNA) from plasma. The developed assay than amplifies in two PCR steps cfHPV-DNA and labels every sample with a unique barcode. After ligating sequencing adaptors, the library can be loaded onto a Flongle or MinION flow cell, depending on the number of barcoded samples used. During sequencing the accompanying software analyzes the generated FASTQ data and quantifies the abundance of cfHPV DNA across a broad dynamic range.

The workflow was designed with ease of use, cost efficiency and possible automation in mind.

Figure 1: *Overall workflow from blood collection to report. The assay includes two 96 well plates with oligonucleotides and reference DNA to process 32 samples per HPV RUO Kit and an accompanying software for real time data interpretation.*



5. Result

Exemplary experiment which shows the performance of the assay at its lower detection limit. In all spiked cfDNA samples, HPV 16/18 copies were quantified within our acceptance range and identified correctly HPV positive & negative samples.

The library consisted of 27 samples and an expected amplicon read length of 284 bp, was sequenced on a MinION flow cell for 24 hours (Kit 14 chemistry, R10.4.1).

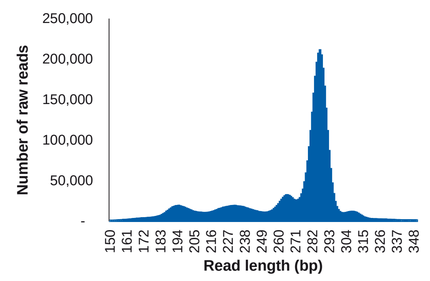


Figure 2: *Raw reads size distribution.*

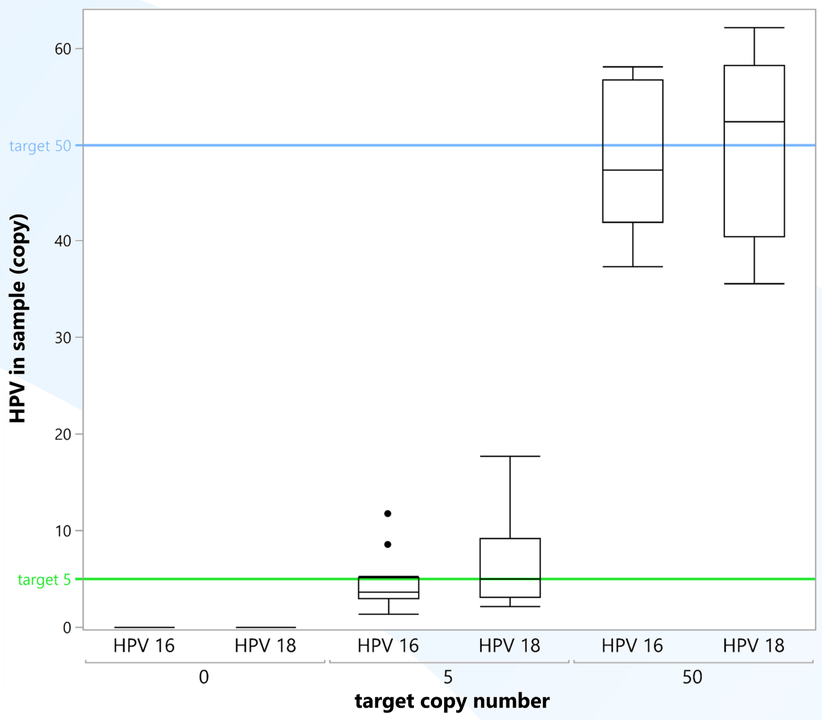


Figure 3: *Performance data of HPV RUO Kit. Quantification of artificially spiked HPV 16/18 DNA in plasma cfDNA. Run data: 17 Mio raw reads (34 Mio after 72 hours), 9.5 Mio dual barcoded reads, 7.4 Mio reads aligned to HPV 16/18. Spiked copy numbers: 0 copy N=12, 5 copy N=10, 50 copy N=5.*

6. Conclusion

With our approach to quantitatively detect HPV in human plasma samples, we present a comprehensive workflow enabling quick turn around times, cost efficient and de-centralized sample analysis. Among other applications this method can be used to monitor tumor burden of HPV associated cancers.

The HPV RUO Kit performs similar to our CLIA validated lab developed test (HPV-SEQ) quantitively detecting HPV 16/18 in plasma cfDNA. The streamlined workflow and use of Nanopore sequencing platform allows tailored sequencing runs for 1 to 2 samples using a Flongle flow cell or up to 32 samples using a MinION flow cell in only 24 h.

References

(1) Sloane, H. et al. (2021) Ultra-sensitive detection and quantification of HPV DNA in the plasma of patients with oropharyngeal squamous cell carcinoma (OPSCC) enrolled in the OPTIMA 2 treatment de-escalation trial. J Clin Oncol. 39:15_suppl, 6048.
(2) Chera, B. S. et al. (2019). Rapid Clearance Profile of Plasma Circulating Tumor HPV Type 16 DNA during Chemoradiotherapy Correlates with Disease Control in HPV-Associated Oropharyngeal Cancer. Clinical Cancer Research, 25(15), 4682–4690.
(3) Hanna, G. J et al. (2018). Plasma HPV cell-free DNA monitoring in advanced HPV-associated oropharyngeal cancer. Annals of Oncology.

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