

# Validation of QuanDX Meltpro High Risk HPV Genotyping Assays

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## Introduction

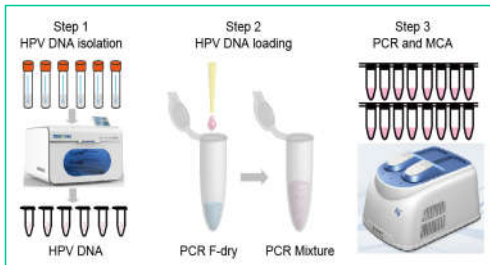
QuanDX is a start up molecular diagnostic company. An HPV detection assay was developed based on technology of multi-color melting curve analysis. This test uses PCR followed by high-resolution melting curve analysis for the detection of 14 high-risk HPV types. High-risk HPV subtypes can be differentiated in a single reaction. This test specifically identifies 14 high-risk HPV types, including HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66, and HPV-68. QuanDX assay was originally developed for qualitative detection of HPV genotyping and cervical cancer screening using cervical cells collected by swabs. We are now validating this test for detecting HPV in different cancers and specimen types.

## Methods

### Principle of Probe-Based Melting Curve Analysis:

HPV test kit is based on the technology of multi-color melting curve analysis (MMCA). After PCR amplification, HPV genotypes are identified by the different  $T_m$  values of hybridizing PCR products with the fluorescent probes. The hybridization probe that fully matches its wild-type target gives the highest  $T_m$  value. However, a mutant-type target paired with the probe gives a lower  $T_m$  value. Mutation is detected as  $T_m$  deviation ( $\Delta T_m$ ) compared to the wild-type hybrid.

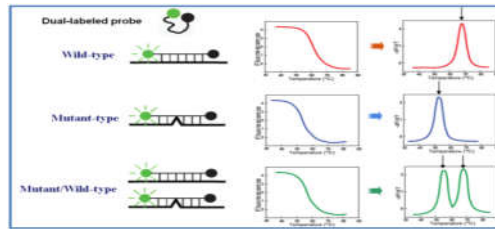
### HPV Assay Set Up



**QuanDX Assay:** Approximately 1000 ng of DNA in 25 ul of water is added to the PCR-dry tube (powder) which contains all the reagents required for the assay. Positive controls and negative controls provided by the company should be used in each assay. The assay will be performed in a SLAN 96 PCR system which is equipped with auto run software that identifies  $T_m$  values of each probe-target hybrid and converts  $T_m$  values to HPV subtypes automatically.

## Methods-2

### Data Interpretation



ROX	HPV-31	49° C $T_m$ < 53° C	HPV-33	53° C $T_m$ < 59° C
	HPV-16	59° C $T_m$ < 64° C	HPV-35	64° C $T_m$ < 68° C
	HPV-68	68° C $T_m$ < 73° C	HPV-18	73° C $T_m$ < 77° C
CY5	HPV-56	45° C $T_m$ < 52° C	HPV-52	52° C $T_m$ < 57° C
	HPV-45	57° C $T_m$ < 63° C	HPV-39	63° C $T_m$ < 70° C
FAM	HPV-59	44° C $T_m$ < 48° C	HPV-66	48° C $T_m$ < 54° C
	HPV-58	54° C $T_m$ < 60° C	HPV-51	60° C $T_m$ < 74° C
HEX	Internal control	50° C $T_m$ < 70° C		

## Results

### Experiment No.1

S.No	Year	Specimen No.	Description	*Other Methods HPV	QuanDX Results HPV
S.1	2015	V7534388	HPV, High-Risk ONLY, In Situ DNA	Pos	HPV-16
S.2	2015	V7534128	HPV High Risk E6/E7, RNA ISH	Pos	HPV-16
S.3	2016	15-363-02137A	HPV, High-Risk ONLY, In Situ DNA	Neg	HPV-16
S.4	2016	16-006-02460A	HPV, High-Risk ONLY, In Situ DNA	Neg	HPV-16
S.5	2016	16-06-2471	HPV, High-Risk ONLY, In Situ DNA	Pos	HPV-16
S.6	2016	16-913-02611	HPV, High-Risk ONLY, In Situ DNA	Neg	HPV-16
S.15	2016	16-064-1924	HPV, High-Risk ONLY, In Situ DNA	Pos	HPV-16
S.16	2016	16-062-2432	HPV, High-Risk ONLY, In Situ DNA	Pos	HPV-16
S.21	2016	16-098-2499	HPV, High-Risk ONLY, In Situ DNA	Pos	HPV-16
S.22	2016	16-102-02668	HPV, High-Risk ONLY, In Situ DNA	Pos	HPV-16
S.23	2016	16-103-02711	HPV High Risk E6/E7, RNA ISH	Pos	HPV-16
S.26	2016	16-119-2496	HPV, High-Risk ONLY, In Situ DNA	Pos	HPV-16
S.29	2016	V7572853	HPV, High-Risk ONLY, In Situ DNA	Pos	HPV-16
S.33	2016	16-165-02511	HPV High Risk E6/E7, RNA ISH	Pos	HPV-16

DNA extracted from FFPE samples of squamous cell carcinomas of head and neck were tested for HPV infection with QuanDX assay. All of them were positive with HPV-16. This may be due to high sensitivity of the assay compared to other methods.

\* Other Methods used previously to detect HPV in Head and Neck are In Situ DNA and RNA ISH.

## Experiment No.2

Sample name	Description	HPV	QuanDX Results
Positive Control			Positive
Negative Control			Negative
TST1-1781	Colon, Adeno	Unknown	Negative
TST1-1774	Met/Mmel	Unknown	Negative
TST1-1762	Lung	Unknown	Negative
TST1-1747	Colon	Unknown	Negative
TST1-1744	Colon	Unknown	Negative
TST1-1731	Met, mel	Unknown	Negative
TST1-1684	Brain	Unknown	Negative
TST1-1692	Glioma	Unknown	Negative
TST1-1701	celeb	Unknown	Negative
TST1-1805	Melanoma	Unknown	Negative
TST1-1824	Glioblastoma	Unknown	HPV-16(Manu-decision);
TST1-1852	Lung	Unknown	Negative
Control Neg		Neg	Invalid sample
Control Pos		Positive	HPV-58; HPV-16; HPV-45;

DNA extracted from different cancer types were tested for HPV infection. All of them were negative except Glioblastoma.

## Experiment No.3 and 4

S.No	Description	HPV	QuanDX Results
Positive Control		Positive	Positive
Negative Control		Negative	Negative
TST1-1676	ANAPLASTIC ASTROCYTOMA	UN	HPV-35
TST1-1683	GLOBLASTOMA	UN	HPV-16
TST1-1687	GLOBLASTOMA	UN	Negative
TST1-1775	GLOBLASTOMA	UN	Negative
TST1-1786	MET MAL MELANOMA	UN	Negative
TST1-1783	Adenocarcinoma	UN	Negative
TST1-1807	GLIOGENDROGLIOMA	UN	Negative
TST1-1815	MET MELANOMA	UN	Negative
TST1-1836	GLOBLASTOMA	UN	Negative
TST1-1855	BRAIN TUMOR	UN	Negative
TST1-1939	MET ADENOCARCINOMA, RENAL ORIGIN	UN	Negative
TST1-1945	MET MELANOMA	UN	Negative
TST1-1955	MET EMBRYONAL RHABDOMYOSARCOMA	UN	Negative
TST1-1777	Adenocarcinoma of lung	UN	Negative

DNA extracted from different cancer types were tested for HPV infection. All of them were negative except Anaplastic Astrocytoma and Glioblastoma

S.No	Description	HPV	QuanDX Results
Positive Control		Positive	Positive
Negative Control		Negative	Negative
TST1-1676	ANAPLASTIC ASTROCYTOMA	HPV-35	HPV-35;
TST1-1683	GLOBLASTOMA	HPV-16	HPV-16;
TST1-1687	GLOBLASTOMA	Negative	Negative
16-006-02460A	HPV, High-Risk ONLY, In Situ DNA	HPV-16	HPV-16;
TST1-1786	MET MAL MELANOMA	Negative	Negative
16-06-2471	Head and neck	HPV-16	HPV-16;
16-062-2432	Head and Neck	HPV-16	HPV-16;
TST1-1815	MET MELANOMA	Negative	Negative
16-041-02569A	Head and Neck	HPV-16	HPV-16;
TST1-1855	BRAIN TUMOR	Negative	Negative
TST1-1824	GLOBLASTOMA	HPV-16	HPV-16;
TST1-1945	MET MELANOMA	Negative	Negative
16-102-02668	Head and Neck	HPV-16	HPV-16;
TST1-1777	Adenocarcinoma of lung	Negative	Negative

HPV positive and negative samples previously identified were blind tested in a separate assay. Results are 100% concordant

## Experiment No.5

sample name	Description	Other methods	QuanDX with 100 ng
Positive			Positive
Negative			Negative
S.1	HPV, High-Risk ONLY, In Situ DNA	P15-13504 Pos	HPV-16;
S.2	HPV High Risk E6/E7, RNA ISH	SP15 13610 Pos	HPV-16;
S.3	HPV, High-Risk ONLY, In Situ DNA	SP15-13997 C7 Neg	HPV-16;
S.4	HPV, High-Risk ONLY, In Situ DNA	SP16-117 B1 Neg	HPV-16;
S.5	HPV, High-Risk ONLY, In Situ DNA	SP16-160 B1 Pos	HPV-16;
S.6	HPV, High-Risk ONLY, In Situ DNA	SP16 499 A9 Neg	HPV-16;
			QuanDX with 10ng
S.1	HPV, High-Risk ONLY, In Situ DNA	P15-13504 Pos	HPV-16;
S.2	HPV High Risk E6/E7, RNA ISH	SP15 13610 Pos	HPV-16;
S.3	HPV, High-Risk ONLY, In Situ DNA	SP15-13997 C7 Neg	Negative
S.4	HPV, High-Risk ONLY, In Situ DNA	SP16-117 B1 Neg	HPV-16;
S.5	HPV, High-Risk ONLY, In Situ DNA	SP16-160 B1 Pos	HPV-16;
S.6	HPV, High-Risk ONLY, In Situ DNA	SP16 499 A9 Neg	HPV-16;
TST1-1676	ANAPLASTIC ASTROCYTOMA*		HPV-35 Negative
TST1-1683	GLOBLASTOMA*		HPV-16 Negative

\*QuanDX Assay with 100 ng

QuanDX assay was performed by using 100 ng and 10 ng of DNA.

## Conclusions

- This test uses PCR followed by high-resolution melting curve analysis for the detection of 14 high-risk HPV types.
- Easy to perform the assay. Detection sensitivity claimed by company is 200 copies HPV per reaction.
- Squamous cell carcinomas of head and neck were tested for HPV infection with QuanDX assay.
- All 20 of the head and neck samples tested were positive for HPV-16.
- Results obtained with different cancer samples except some glioblastomas were negative for HPV.
- When previously tested HPV positive and negative samples ((by QuanDx Assays) were blind tested, the results were 100% concordant.
- Assay can be performed with 100 ng of DNA (however, lower limit of detection can be determined later)