HPV Genotyping of Solid Tumors Using Real-Time PCR and Multi-Color Melt Curve Analysis

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ABSTRACT

Introduction: Oncogenic Human Papillomavirus (HPV) genotypes are linked to over 600,000 cancer cases worldwide. While the role of HPV in nearly all cervical cancers is established and screening methods are in place, the status of HPV in other cancers has only recently become apparent. HPV-positive OPSCCs and other anogenital cancers have a more favorable prognosis and response to therapy than similar HPV-negative cancers. As such, HPV-status has significant prognostic and treatment implications. To this end, we validated the use the MeltPro High Risk and Low Risk HPV Genotyping Kits from QuanDx® to assess HPV status on OPSCCs and other solid tumors relative to the Roche Linear Array®.

Methods: DNA from 29 formalin fixed paraffin embedded (FFPE) tissues was isolated using the Qiagen Gentra® Tissue Kit. Samples previously tested on the Roche Linear Array® were diluted in water to 25 µL for total DNA ranges from 5-1000 ng. Samples were then added to lyophilized High-Risk or Low-Risk MeltPro Kits and run on a SLAN-96 real-time PCR instrument (QuanDx/Zeesan Biotech, San Jose, CA) per kit instructions over 2.5 hours. Instrument software provided sample genotypes via multi-color melting curve analysis (MMCA); these results were then compared to those obtained from the Roche Linear Array®.

Results: For all 44 reactions from 11 samples run on the Low Risk HPV MeltPro Kit there was complete concordance between assays across all DNA dilutions. The MMCA was well within temperature ranges set by the manufacturer and HPV6 and HPV11 were distinguished from all negative and high risk samples. For 45 reactions from 18 samples, the High Risk HPV MeltPro Kit was 97.8% concordant; however, upon repeat of this sample, the correct results were obtained for all samples. The QuanDX® kits correctly called all negative samples, low risk HPV6 and HPV11 together with high risk HPV genotypes: 16, 18, 33, 35, 45, 51, 52, and 59.

Conclusions: The QuanDX® High-Risk and Low-Risk MeltPro Kits offer an accurate genotyping solution relative to the Roche Linear Array®. Moreover, in our comparison of the workflows between the two assay types, the MeltPro Kits require dramatically less hands on time with shorter turnaround times.

BACKGROUND

While the role of HPV in nearly all cervical cancers is established and screening methods are in place, the status of HPV in other cancers has only recently become apparent. Oral HPV infection confers ~50% fold increased risk for oropharyngeal squamous cell carcinomas (OPSCCs). HPV-positive OPSCCs and other anogenital cancers have a more favorable prognosis and response to therapy than similar HPV-negative cancers resulting higher overall survival (Figure 1). As such, HPV-status has significant prognostic and treatment implications to the extent that beginning in 2018 HPV-associated tumors will have a separate TNM staging.

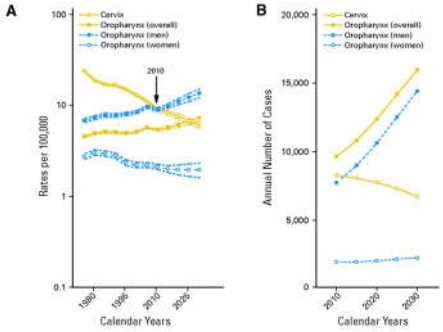

Figure 2. (Anil K. Chaturvedi *et al.* 2011).

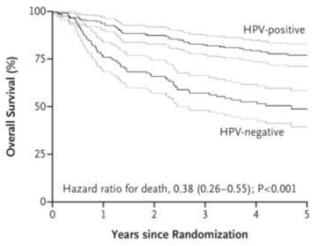

Figure 1. (Maura Gillison *et al.* 2012).

Moreover, while tobacco-related head and neck cancers have declined, HPV OPSCCs have risen significantly (225% from 1998 to 2004), primarily among men. In this context, it is forecasted that the incidence of HPV OPSCC in men will surpass cervical cancer by 2020 and that OPSCCs will constitute the majority of all head and neck cancers by 2030 (Figure 2).

In summary, being able to accurately identify HPV genotypes in patients is of critical importance. To this end, we sought to validate a molecular test to assess HPV status on OPSCCs and other solid tumors relative to the Roche Linear Array®. Our aim is to find a similarly sensitive test that is both cost and time sensitive.

METHODS

Samples: DNA from 29 formalin fixed paraffin embedded (FFPE) tissues was isolated using the Qiagen Gentra® Tissue Kit. DNA concentrations were determined spectrographically and dilutions made from 5-1000ng in 25 µl nuclease free water.

HPV Genotyping: For either High-Risk or Low-Risk MeltPro Kits, amplicon specific probes with genotype-specific melting temperatures were used for both an internal control and HPV genotype determination (Figure 3).

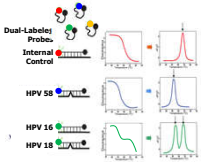

Figure 3: Probe based chemistry and melt curves (adapted from QuanDx®)

Channel	HR Genotype	Tm (°C)
ROX	HPV-31	49 ≤ Tm < 55°
	HPV-33	53 ≤ Tm < 59°
	HPV-16	59 ≤ Tm < 64°
	HPV-35	64 ≤ Tm < 68°
	HPV-68	68 ≤ Tm < 73°
CY5	HPV-18	73 ≤ Tm < 77°
	HPV-56	49 ≤ Tm < 52°
	HPV-52	52 ≤ Tm < 57°
	HPV-45	57 ≤ Tm < 63°
	HPV-39	63 ≤ Tm < 70°
FAM	HPV-59	44 ≤ Tm < 48°
	HPV-66	48 ≤ Tm < 54°
	HPV-58	54 ≤ Tm < 60°
	HPV-51	60 ≤ Tm < 74°
HEX	Internal Control	90 ≤ Tm < 79°

Table 1: High-risk HPV Tms.

Channel	LR Genotype	Tm (°C)
ROX	HPV-42	49 ≤ Tm < 54°
	HPV-89	54 ≤ Tm < 62°
	HPV-81	62 ≤ Tm < 66°
	HPV-43	66 ≤ Tm < 71°
	HPV-40	71 ≤ Tm < 77°
CY5	HPV-72	48 ≤ Tm < 53°
	HPV-44	53 ≤ Tm < 57°
	HPV-61	57 ≤ Tm < 63°
	HPV-84	63 ≤ Tm < 67°
	HPV-70	67 ≤ Tm < 73°
FAM	HPV-11	55 ≤ Tm < 62°
	HPV-6	66 ≤ Tm < 69°
	HPV-53	69 ≤ Tm < 85°
	HPV-26	85 ≤ Tm < 79°
HEX	Internal Control	90 ≤ Tm < 79°

Table 2: Low-risk HPV Tms.

Samples were added to either lyophilized High-Risk or Low-Risk MeltPro Kits and run on respective thermal programs on a SLAN-96 real-time PCR instrument (QuanDx/Zeesan Biotech, San Jose, CA). Amplification and melt-curve analysis completes in 2.5 hours. Software determined results were confirmed manually using amplification, and melt-curve data. All genotype-specific Tm's were confirmed with those given by the manufacturer for the high-risk and low-risk kits respectively (Tables 1 and 2). These results were then compared to those obtained from the Roche Linear Array®.

RESULTS

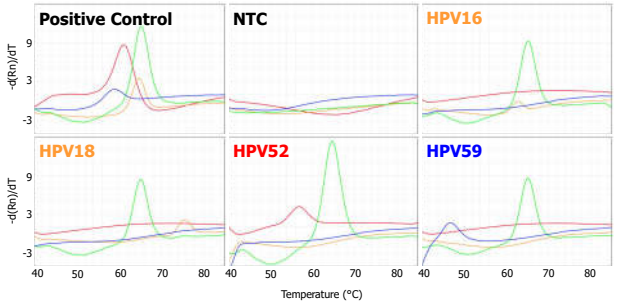

Figure 4. Melt peak analysis for controls and HPV strains representing common High-risk genotypes (HPV: 16, 18, 52, and 59).

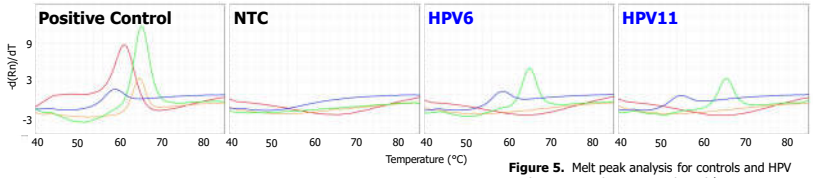

Figure 5. Melt peak analysis for controls and HPV strains representing common Low-risk genotypes HPV6 and HPV11.

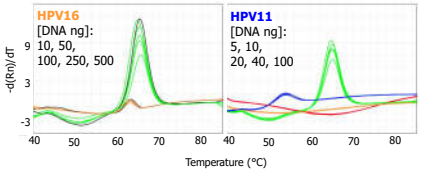

Figure 6. Reproducibility of High-risk (HPV16) and Low-risk (HPV11) genotypes across DNA concentrations.

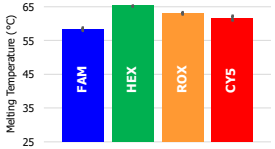

Figure 7. Positive control melt peak reproducibility (N=5)

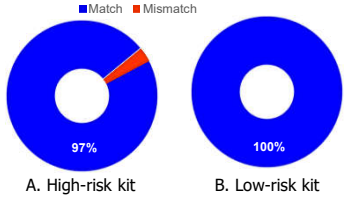

Figure 8. Concordance HPV genotypes as determined by Linear Array with: (A) 18 samples run with the High-risk Meltpro Kit and (B) 11 samples run with the Low-risk Meltpro kit.

CONCLUSIONS

The High-risk and Low-risk MeltPro Kits from QuanDx® accurately and consistently identify HPV genotypes from FFPE solid tumor samples. To expand on this we have sought additional samples and collaboration with outside labs, as well as begun a validation of our automated DNA extraction. In combination, the MeltPro kits offer an accurate HPV genotyping solution that requires both dramatically shorter hands on and turn around times relative to the Roche Linear Array®.

- Test accuracy was near 100% and corrected upon repeat.
- Test intra and inter-run reproducibility and repeatability was found to be high with little variation between melting temps.
- Input DNA concentrations is exceedingly low and well within ranges obtained from most FFPE samples.

ACKNOWLEDGEMENTS & REFERENCES

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