Detection of BRAF V600 Mutations in Tissue from Various Tumor Sites: Validation of VELA Diagnostics Sentosa® SA BRAF V600 PCR Test

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Introduction

When mutations accumulate in genes that control essential processes such as cell proliferation, differentiation, and death, the delicate balance of these processes becomes disrupted and can lead to cancer (1). Various types of cancer arise from these gene mutations; a common mutation among colorectal, ovarian, papillary thyroid carcinoma and melanoma occurs in the BRAF gene. BRAF is a serine-threonine kinase and when mutated can have kinase activity 10 times greater than normal. The constitutively active BRAF kinase phosphorylates and activates the MAP/ERK signaling pathway resulting in uncontrolled growth and tumor formation (1, 2).

The most common activating BRAF mutation occurs in exon 15 at nucleotide 1799, representing approximately 90% of BRAF mutations in the aforementioned cancer types. In this mutation termed BRAF V600E c.1799T>A [p.Val600Glu], glutamate is substituted for valine at codon 600. Occurring much less frequently, approximately 6-12% of the time, is the BRAF V600K mutation, c.1798_1799delCTinsAA [p.Val600Lys] where lysine is substituted for valine at codon 600; although rare in colorectal, ovarian, and papillary thyroid cancers, is often observed in melanomas (1,3). Other activating BRAF mutations are known but the two mentioned here are of primary pathological significance.

The decision to institute V600 BRAF testing in-house was test-volume and pathologist driven; tumor response to EGFR and other BRAF-targeted therapies is their main focus for testing. Generally, the presence of an activating BRAF mutation at V600 correlates with a poor prognosis for patients because the tumors are typically non-responsive to EGFR inhibitors. In contrast, the presence of an activating BRAF mutation in melanoma is associated with response to BRAF-targeted therapy (4). Pathologists direct targeted drug therapies based on the results obtained from the presence or absence of specific mutations such as BRAF. We currently perform real-time PCR for the V600E activating BRAF mutation on the Roche LightCycler platform using lab-developed primers and probes.

The aim of this study is to increase our test sensitivity, specificity and decrease hands-on-time and the number of tests being sent out to reference laboratories. To achieve this we compared our current lab-developed BRAF PCR to the Sentosa® SA BRAF V600 PCR assay developed by VELA Diagnostics.

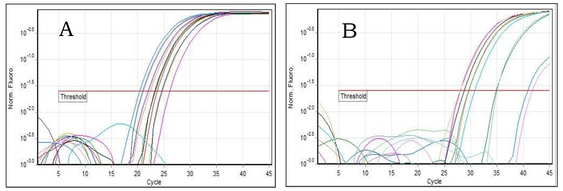
Materials and Methods

A collection of 130 specimens were included in this study; specimens were either formalin-fixed paraffin embedded (FFPE) tissues or FNA thyroid specimens in ThinPrep PreserveCyt® Solution. Extractions were performed using the Qiagen QIAamp® DNA FFPE Tissue Kit. Each specimen was subject to the lab-developed BRAF V600E real-time PCR assay on the Roche LightCycler 480 as well as automated PCR preparation on the SentosaTM SX101 platform and PCR amplification on the VELA Rotor-Gene Q, using the SentosaTM SA BRAF V600 PCR reagents from VELA Diagnostics.

Discrepant samples were repeated on both assays and pyrosequencing was performed using the Qiagen PyroMark Q24 to determine the presence or absence of the V600K mutation. Based on the patients' clinical condition, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for both assays. For 8 of the melanoma specimens mutation analysis was performed by next generation sequencing using the SentosaTM SQ Melanoma Panel. Library preparation was performed on the fully automated SentosaTM SX101 platform, followed by template preparation and sequencing on the SentosaTM SQ301.

Results

Figure 1. Data from the Sentosa® SA BRAF V600 PCR Assay.



A) amplification curves for the internal control (IC) for each specimen. B) amplification curves for the V600 target of the BRAF gene. To determine positivity of a specimen the IC crossing value must be subtracted from the target crossing value; if the difference is ≤12 a V600 mutation was detected.

Table 1. Summary of BRAF V600E mutation detection compared to patient clinical condition, using the NorDx lab-developed real-time PCR assay.

NorDx V600E PCR Result	Clinical Condition		
	BRAF Mutant	BRAF Wild Type	Total
Positive Mutant	50	2	52
BRAF Wild Type	6	72	78
Total	56	74	130

Table 2. Summary of BRAF V600E and V600K mutation detection as compared to patient clinical condition, when performing the Sentosa® SA BRAF V600 real-time PCR assay.

VELA V600 PCR Result	Clinical Condition		
	BRAF Mutant	BRAF Wild Type	Total
Positive Mutant	55	1	56
BRAF Wild Type	1	73	74
Total	56	74	130

Tables 1 & 2. Overall there was 94% agreement between the two BRAF PCR assays. The 6% disagreement can be attributed, mostly, to the presence of V600K mutations, which are detectable by the VELA PCR but not the NorDx PCR.

Table 3. Performance characteristics for the NorDx lab-developed BRAF PCR assay compared to the VELA Diagnostics Sentosa® SA BRAF V600 PCR assay

	NorDx V600E PCR	VELA V600 PCR
Sensitivity	*89.30%*	*98.20%*
Specificity	*97.30%*	*98.60%*
PPV	*96.20%*	*98.20%*
NPV	*92.30%*	*98.60%*

Table 3. Following discrepant sample resolution, the sensitivity of the NorDx PCR as compared to the VELA PCR (89.3% and 98.2% respectively) was nearly 10% less, expectedly so due to that assay's inability to detect BRAF V600K. Specificity for both tests was greater than 97%.

Results (cont.)

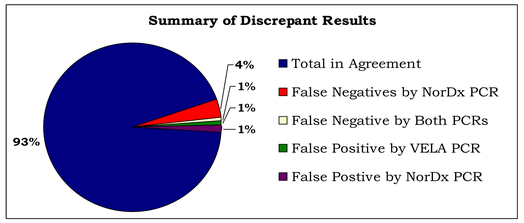


Figure 2. Summary of discrepant BRAF V600 results. 93% of specimen results were in agreement between the two PCR assays. 4% were negative by NorDx PCR and positive by VELA PCR, primarily due to the inability of the NorDx assay to detect the V600K mutation. The remaining discrepant results occurred at a frequency of 1% each; false negatives by both PCR assays, false positives by VELA PCR, and false positives by NorDx PCR.

Table 4. Summary of NGS Results from 8 melanoma specimens used in the BRAF assay comparison

Specimen	PCR Result	PCR Crossing Value	NGS Result	Variant Frequency (%)
1	BRAF POS.	28.32	BRAF V600E	58.12
2	BRAF POS.	29.80	BRAF V600E	54.05
3	BRAF POS.	29.81	BRAF V600E	41.36
4	BRAF NEG.	n/a	BRAF NEG.	n/a
5	BRAF NEG.	n/a	BRAF NEG.	n/a
6	BRAF NEG.	n/a	BRAF NEG.	n/a
7	BRAF NEG.	n/a	BRAF NEG.	n/a
8	BRAF NEG.	n/a	BRAF NEG.	n/a

Table 4. Summary of melanoma specimens tested by PCR and next generation sequencing (NGS). All 8 BRAF results were in agreement between the PCR and the NGS assays; 3 were positive for V600E and 5 were negative for any BRAF mutation. Positives had the mutation occurring at a frequency <40% in the tumor DNA.

Table 5. Summary of non-BRAF variants identified in 8 melanoma specimens using NGS

Specimen	NGS Result	Other NGS Target Variants	Non-Target Variants
1	BRAF V600E	n/a	BRAF p.E611D GNAQ p.G208A
3	BRAF V600E	n/a	CDKN2A p.D84N CDKN2A p.P70L
4	BRAF NEG.	NRAS Q61K	n/a
5	BRAF NEG.	NRAS Q61R	n/a
6	BRAF NEG.	NRAS Q61L	CDKN2A p.R80
8	BRAF NEG.	NO VARIANT	KIT p.R634Q

Table 5. Summary of target variants other than BRAF, as well as non-target variants of interest identified using NGS on the Sentosa® SQ301 Sequencing Instrument. The Sentosa® SQ Melanoma Panel identifies select mutations in the BRAF, NRAS, CDKN2A, MAP2K1, FGFR3, AKT3, KIT, PIK3CA, GNAQ, and GNA11 genes.

Results (cont.)

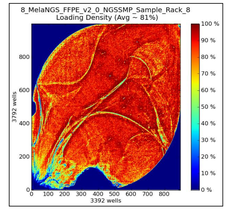


Figure 3. Loading density image for the NGS chip used to run the 8 melanoma samples. Loading was 81.34%, enrichment was 100% with a ratio of 60% clonal: 40% polyclonal beads. Average valid reads per sample was 474,000 and average coverage was 21,000.

Conclusions

In our comparison of the two BRAF PCR assays we determined that the increased automation afforded by the Sentosa® SX101, the lower limit of detection (1.25% mutant), and the increased sensitivity over our homebrew assay, were advantageous for our laboratory. These improvements aid in directing drug therapies for patients who may not be responding to treatment.

Implementing this new technology to detect the activating BRAF V600K mutation has positive implications for the health of our patients, especially those with PTC and melanoma. The response rates of patients with positive V600K tumors to BRAF inhibitors have been documented in several clinical trials. This study also demonstrated the utility of next generation sequencing platforms in melanoma cases without a BRAF mutation.

Continued investigation into the pattern and activity of BRAF mutations and the associated implications is necessary for early detection, effective treatment, and increasing patient survival time through the practice of personalized medicine.

References

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Acknowledgements

Special thanks to VELA Diagnostics for providing reagents and support for this study.