ANALYTICAL VALIDATION OF A SALIVA COLLECTION AND DNA EXTRACTION PROTOCOL FOR A 25-GENE HEREDITARY CANCER PANEL

Jared Cassiano, MS; Jack Landon, BS; Elizabeth Goossen, MS; Debora Mancini-DiNardo, PhD; Benoît Leclair, PhD; Benjamin B. Roa, PhD

Myriad Genetics, Inc. , Salt Lake City, UT

BACKGROUND

- Early identification of individuals carrying genetic mutations in cancer pre-disposing genes strongly impacts clinical management, significantly reducing morbidity and mortality from associated cancer syndromes.
- Analysis of DNA extracted from blood is the gold standard of germline molecular diagnostic testing. However, blood venipuncture can present challenges in some cases. DNA extracted from saliva can prove to be a viable alternative to venipuncture.
- Here, we describe validation data for the collection and DNA extraction of saliva for use with a pan-cancer panel test,[1] an NGS-based sequence and Large Rearrangement (LR) detection platform for a panel of 25 genes associated with 8 cancer syndromes.

METHODS

- 138 paired blood/saliva Population Control samples from healthy individuals and 29 saliva samples from previously tested patients who carry LRs in various genes (24 deletions, 3 duplications, 1 insertion, 1 triplication) were obtained from consented individuals.
- The pan cancer panel test included *APC*, *ATM*, *BARD1*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A* (*p16INK4A* and *p14ARF*), *CHEK2*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *SMAD4*, *STK11*, and *TP53*.
- Sequencing and large rearrangement testing were performed on all genes in the panel except *EPCAM*, where only large rearrangement analysis of terminal exons was performed.
- NGS was performed with PCR-based target-enrichment (RainDance) and sequencing on NextGen sequencers (Illumina). Long Range amplicons were used as surrogate NGS sequencing templates for areas of *PMS2* and *CHEK2* affected by pseudogenes.
- A combination of commercial and laboratory-developed software was used for NGS data processing. Clinically significant variants were confirmed, and regions not meeting minimum NGS quality or coverage metrics were independently tested, with orthogonal site-specific Sanger sequencing.
- Gene dosage analysis was performed with NGS across gene coding exons and certain functionally characterized promoter regions.
- LR positives were confirmed by repeat testing with NGS dosage analysis, semi-quantitative PCR, MLPA and/or microarray-CGH analysis (aCGH). For *PMS2* and *CHEK2*, LR mutation positives were evaluated with MLPA plus orthogonal methods, where appropriate.
- All results for paired Population Control samples were compared between saliva and blood DNA counterparts.

RESULTS

- A total of 11,743,250 wild-type bases and 5,380 sequence variants (includes SNVs, small insertions / deletions) (Table 1) were detected in 138 saliva DNA samples at a minimum read depth of 50X.
- All wild-type and variant NGS sequencing results were 100% concordant between paired blood / saliva DNA counterparts.
- All LR mutation positive saliva samples showed 100% concordance with LR results based on previous testing of blood samples (Table 2).
- 100% concordance was also observed for all saliva samples using orthogonal LR confirmation methods (aCGH, MLPA, semi-quantitative PCR).

TABLE 1. Sequence Variants Detected During Pan-Cancer Panel Testing Using Saliva Samples

Gene	N
APC	578
ATM	302
BARD1	582
BMPR1A	159
BRCA1	634
BRCA2	535
BRIP1	342
CDH1	319
CDK4	0
CHEK2	11
MLH1	180
MSH2	167
MSH6	273
MUTYH	154
NBN	413
CDKN2A (p14ARF)	11
CDKN2A (p16INK4a)	11
PALB2	61
PMS2	381
PTEN	1
RAD51C	6
RAD51D	65
SMAD4	1
STK11	56
TP53	138
TOTAL VARIANTS	**5380**
Number of Samples	138
Variants / Sample	38.99

TABLE 2. Large Rearrangements Detected During Pan-Cancer Panel Testing Using Saliva Samples

Gene	Called LR
BRCA1	del exon1
	del exons 1-2
	del exons 1-3
	del exons 8-13
	del exons 8-24
	del exons 21-24
	del exon 24
	exon 13 ins6kb
BRCA2	triplication of exons 14-24
BRIP1	del exons 1-2
	del exons 10-12
CHEK2	dup exons 2-14
EPCAM,MSH2	3' terminal deletion, del exons 1-7
MLH1	dup exons 6-12
MSH2	del exons 1-6
	del exons 14(3')-15
MSH6	del exons 2-6
NBN	del entire gene
PALB2	del exons 7-13
	del exon 8
	del exons 12-13
PMS2	del exons 6-9
	del exons 9-10
	del exon 10
RAD51C	del exon 3
	del exon 3(3')
RAD51D	del exon 1
	del exons 1-8

DISCUSSION

- Our pan-cancer NGS test platform has proven robust for saliva samples in accurately calling nearly 12 million bases in this validation. The platform performed equally well with LR detection. No discrepancies between the saliva and their blood comparators were detected.
- The pan-cancer test validation data for the saliva sample type yielded an analytical sensitivity of 100% (99.93% - 100%, 95% C.I.).
- The total sequencing validation data, which identified 5,380 sequence variants in this study combined with 3,923 sequence variants previously validated on blood derived samples[1], affords an analytical sensitivity of 100% (99.96% - 100%, 95% C.I.) and analytical specificity of 100% (99.99% - 100%, 95% C.I.).[2]
- The ability to detect both sequencing variants and large rearrangement mutation positives with NGS across a 25-gene panel provides better efficiency and response time for the patient.
- The development and validation of the saliva sample type protocol for use with pan-cancer panel testing expands accessibility to the test, especially when blood venipuncture is a challenge.

REFERENCES

1. Judkins T, et al. Development and analytical validation of a 25-gene next generation sequencing panel that includes the BRCA1 and BRCA2 genes to assess hereditary cancer risk. *BMC Cancer.* 2015 Apr 2;15:215.
2. Myriad Genetic Laboratories: myRisk Technical Specifications. https://s3.amazonaws.com/myriad-library/technical-specifications/myRisk+Hereditary+Cancer+Tech+Specs.pdf

Presented at ASHG - October 8, 2015