Presented at ASHG - October 8, 2015

**BACKGROUND**

- Early identification of individuals carrying genetic mutations in cancer predisposing 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, an NGS-based sequence and Large Rearrangement (LR) detection platform for a panel of 25 genes associated with 8 cancer syndromes.
- The pan-cancer panel test included APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRI1, CDH1, CDK4, CDKN2A (p16INK4A and p14ARF), CHEK2, MLH1, MSH2, MSH6, MUTHY, NBN, PALB2, PMS1, 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 a 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.

**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, BRI1, CDH1, CDK4, CDKN2A (p16INK4A and p14ARF), CHEK2, MLH1, MSH2, MSH6, MUTHY, NBN, PALB2, PMS1, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, and TP53.
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- 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).

**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, affords an analytical sensitivity of 100% (99.96% - 100%, 95% C.I.) and analytical specificity of 100% (99.99% - 100%, 95% C.I.).
- 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**


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