G04. Long-Range PCR for Clinical Mutation Detection of *SFTPA1* and *SFTPA2* Genes

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Introduction: Germ line mutations in Surfactant Protein A (SP-A) are correlated with severe pulmonary disease. SP-A exists as two isoforms, SPA-1 (*SFTPA1*) and SPA-2 (*SFTPA2*); these surfactants serve two main roles in the lungs: alveolar gas exchange and innate immune defense. Both genes are located on the q arm of chromosome 10 and have >98% and >90% sequence identity in their coding and non-coding regions, respectively. The high identity of the two genes makes traditional PCR design and DNA sequencing exceptionally challenging. We have developed a genetic DNA sequencing test based on long-range PCR for the clinical detection of mutations in *SFTPA1* and *SFTPA2*. **Methods:** Primer pairs were designed to specifically amplify approximately 10 kb of genomic sequence for both *SFTPA1* and *SFTPA2*. PCR amplification using Takara 'One Shot LA PCR' was performed for each gene on genomic DNA extracted from whole blood. The two PCR products were purified using Qiagen's 'QIAquick PCR Purification microcentrifuge' kit. Each PCR product underwent restriction enzyme digestion with EcoRV to confirm the specific identity of *SFTPA1* or *SFTPA2*. The PCR products were sequenced using internal primers with Applied Biosystems (AB) BigDye Terminator 3.1 and purified using AB BigDye Xterminator kit. Capillary electrophoresis of the sequenced fragments was performed on an AB 3130xl to generate chromatograms. The chromatograms were analyzed and interpreted using AB Sequence Analysis, FinchTV (Geospiza), Mutation Surveyor (Softgenetics), and Alamut (Interactive Biosoftware). **Results:** Gel electrophoresis demonstrated the distinct banding patterns that are specific for *SFTPA1* and *SFTPA2* PCR products digested by EcoRV. Bi-directional DNA sequence was obtained for all coding sequence with quality scores in Mutation Surveyor greater than 20.The chromatograms showed gene-specific alleles at areas of non-homology for all four exons in the two genes. Samples with previously reported mutations were accurately called by the mutation software. **Conclusions:** Although long-range PCR is not typically used for clinical sequencing and mutation detection, it is useful for genes such as *SFTPA1* and *SFTPA2*, which have high similarity and limited unique sequences for priming. In the research setting, investigators of these genes have been limited to priming sites that also contain single-nucleotide polymorphisms of unknown significance. Using long-range PCR, we are able to avoid priming sites that contain single-nucleotide polymorphisms and specifically amplify *SFTPA1* and *SFTPA2* in individual reactions. As additional clinically significant genes are discovered that have isoforms possessing high sequence identity, long-range PCR may have a more prominent role in clinical DNA sequencing tests.

G42. A Multi-Gene Assay Coupled with Bioinformatics: An Interdisciplinary Approach to Pharmacogenetics Testing

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Introduction: The rationale for the “Pharmacogenomic Resources for Enhanced Decisions in Care and Treatment” (PREDICT) initiative at our institution is to provide real-time genotype analysis and decision support to facilitate individualized drug therapy. Implementation of this initiative involved adoption of a mid-throughput multi-gene assay in the clinical laboratory. An interdisciplinary team approach was required and a bioinformatics infrastructure was essential for quality control (QC) reports and delivering patient results to healthcare providers in a timely, comprehensive, and secure manner. **Methods:** 2000 DNA samples primarily from patients in the catheterization lab were referred for testing using the VeraCode ADME (Absorption, Distribution, Metabolism, Excretion) Core Panel Kit (Illumina) and the BeadXpress plate reader (Illumina). The kit interrogates 185 loci across 34 genes. Cell line control DNA with known genotypes was used as positive controls on each 32 specimen plate. **Results:** Genotype results on 34 genes were generated, reviewed and securely stored. Currently, only *CYP2C19* variants are reported with associated decision support for Clopidogrel dosage. Sophisticated bioinformatics generate

comprehensive QC reports to monitor for sample reproducibility, variant allele frequencies and locus performance. Reproducibility for Paragon and Coriell control DNA cell lines at 185 loci was 99.58% and 98.34%, respectively. A monthly QC plate was initiated to monitor the reproducibility of patient results by performing random repeat testing of patient specimens from the preceding month and checking for concordant results at all 185 loci. Thus far, 64 samples have been retested, with 98.67% concordance across all loci and 100% concordance for *CYP2C19* variants. Observed *CYP2C19* allele frequencies are similar to that previously reported from the Database of Single Nucleotide Polymorphisms (dbSNP). 15/185 loci are consistently "poor performers" failing to provide results in >95% of patient samples analyzed on each plate. **Conclusions:** Implementation of clinical testing for pharmacogenetic variants utilizing this assay requires stringent review of the results. The establishment and monitoring of multiple QC indicators is essential and helps to ensure the accuracy of the results reported by this test. The established bioinformatics infrastructure coupled with the genotype data generated from the ADME kit enables genetically informed medicine and allows expansion of the decision support process for drug dosing of other known allelic variants for genes included in this kit.

ID44. Lack of a BK Virus DNA International Standard Complicates Comparison of Results Between Laboratories and Development of a Lab-Developed Quantitative Assay

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Introduction: BK virus (BKV)-induced nephropathy causes allograft loss in approximately 5% of renal transplant patients. Risk of allograft loss increases with high viral load (VL); monitoring plasma VL is used to detect viremia and allow for immunosuppression reduction to prevent allograft loss. Cut-off values for use in this monitoring paradigm have been recommended but have been difficult to implement due to quantification variability between different assays in the absence of an international standard that can be used as a uniform calibrator. Assay-dependent quantitative variability is particularly problematic for laboratories transitioning from reference laboratory testing after developing their own assays and for patients monitored at multiple laboratories. **Methods:** Split plasma samples (n= 136) were tested by the Qiagen artus BK Virus RG PCR Kit (artus) and at least one other method including Eragen Multi-code BK Primer ASR (Eragen), a reference laboratory (RefLab1) using Nanogen ASR reagents, reference lab 2 (RefLab2) using Focus ASR reagents, or in-house testing using Focus ASR reagents. Calibrators included artus QS standards, Zeptometrix BK Virus Linearity Panel, Acrometrix Quantification Panel, serially diluted Applied Biotechnologies BK Viral DNA (ABI), and linearized plasmids (RefLab1 and RefLab2 only). Selected samples were genotyped by direct sequencing. **Results:** BK VL measured by artus were about 1-log lower than RefLab1, with excellent linear correlation; similar bias was observed with commercial panels. Plasmas (N=40) were tested by 4 methods: artus using 4 different standard curves, Eragen with Zeptometrix standard curve, RefLab1, and RefLab2. No bias was observed between artus and Eragen results or between RefLab1 and RefLab2. However, a 0.5-2.2 log bias was observed between artus and both reference labs. The artus results quantified using different standard curves were similar, except with ABI calibrators that showed a bias of 0.5-log. A subset of samples was genotyped by sequencing including those with the largest and smallest observed bias. Samples with genotypes 1a, 1b1 and 1b2 showed a 1-log bias between artus and RefLab1; while no quantification bias was observed for genotype 4 suggesting that RefLab1 method under-quantifies genotype 4. **Conclusions:** Comparison of several BKV DNA quantification methods demonstrated quantitative bias, but overall good correlation. Quantitative bias was likely due to the use of different calibrators for each assay although some assay designs may under-quantify genotype 4. The observed bias highlights the need for BKV international standards. Inability to standardize quantification complicates validation of laboratory developed tests and clinical management of patients monitored in multiple labs.