Title: Preliminary Studies of Using Matrix-Assisted Laser Desorption/Ionization–Time of Flight for Detection of *CFTR* Variants

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Introduction: Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) is a targeted method to detect known genetic variants. Using MALDI-TOF, we initially developed an assay for 119 variants in the *CFTR* gene. A preliminary assay was designed, tested for feasibility internally and sent to two volunteer laboratories for evaluation.

Methods: Assays for the targeted variants were designed using the online Agena Assay Design Suite (ADS, v2.0, San Diego CA). Primer sequences were aligned in silico against the GRCh38 reference genome.

Genomic DNA is subjected to multiplex PCR amplification followed by shrimp alkaline phosphatase (SAP) to remove unincorporated dNTPs. In a subsequent reaction, probes that are adjacent to the variant of interest anneal to the PCR products and undergo single base extension. The detection platform uses matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry technology to determine the extension oligonucleotides mass and differentiate variants.

Testing was performed by 2 external laboratories, including samples provided by a third laboratory, to determine accuracy, analytical sensitivity and specificity from orthogonally tested samples (e.g. next generation sequencing and MASSArray.

Results: The external laboratory results (82 samples) were 100% concordant with previously tested results for 100% accuracy. Three samples failed testing. Fifty-two chromosomes where the reference was positive tested positive and 78 chromosomes where the reference was negative tested negative. Figure 1 includes the variants included in the orthogonal testing. The analytical sensitivity is 100% (95% CI; 93.1-100%) and the analytical specificity is 100% (95% CI; 95.3-100%).

Conclusions: MALDI-TOF is a targeted method to detect known variants. It has mid-range multiplexing capabilities allowing the detection of approximating 25 variants/well. As such, we developed a targeted assay to detect over 100 *CFTR* variants. The assay was designed to detect the 100 variants as recommended by ACMG, plus additional variants that were recommended by a voice of the customer survey.

Orthogonal studies performed in the external laboratories were 100% concordant with the previous results. A total of 82 DNAs were tested. The most common variant in the data set, excluding 5T, was as expected F508del (n=7), followed by R117H (n=4). Note that the samples included in the orthogonal testing were not random and chosen to include as many variants as possible.