**Metabotypes of Subjects with Adverse Reactions Following Vaccination: A Pilot Study**

A total of 200 study samples were thawed on ice for sample preparation, 400 uL of the thawed serum sample were transferred to labeled tubes on ice where they were mixed with 1200uL of MeOH. Analytical quality control (QC) phenotypic pooled samples (3/Group) were generated by transferring pre-determined volumes of each sample from each respective phenotypic Group’s experimental samples into four different 2.0 mL LoBind tubes. The Phenotypic Pool tubes were vortexed, and 3 aliquots of 400 uL was transferred to Phenotypic Pool tubes for each Group. In addition, a study pool was generated by transferring 200 uL of serum from 25 randomly selected experimental samples into a 10.0 mL tube, vortexed and aliquoted into 10 Study Pool tubes. Methanol was added to all tubes (1200 uL), sample tubes were vortexed for 2 min on a multi-tube vortexer and centrifuged at 16,000 rcf for 5 min. A 1000 µl aliquot of the supernatant was transferred into new pre-labeled 2.0 mL LoBind tubes and lyophilized to complete dryness overnight. Samples were reconstituted with 700 uL of NMR Master Mix solution containing Chenomx ISTD: DSS-d6 and D2O-Phosphate Buffer at 7.4 pH. The tubes were vortexed for 4 min on a multi-tube vortexer and centrifuged at 16,000 rcf for 5 min. A 600 uL of each sample supernatant was transferred into a pre-labeled 5mm 4" NMR tubes for data acquisition on a 700 MHz spectrometer.

1H NMR spectra of serum samples were acquired on a Bruker Avance 700 MHz NMR spectrometer (located at the David H. Murdock Research Institute) using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25 ℃. A 1D NOESY pre-saturation pulse sequence (noesypr1d, [recycle delay (RD)-90°-t1-90°-tm-90°-acquire free induction decay (FID) was used for data acquisition. For each sample 128 transients were collected into 65k data points using a spectral width of 8.417 kHz (12.0227 ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 3.893 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. NMR spectra were processed using TopSpin 3.2 software (Bruker-Biospin, Germany). Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5 Hz. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the DSS-d6 signal. The quality of each NMR spectrum was assessed for the level of noise and alignment of identified markers. Spectra were assessed for missing data and underwent quality checks. NMR data was binned (0.70-8.50ppm) after excluding DSS- d6, methanol (3.30-3.37ppm) and water (4.66 – 5.16ppm), and integrated using intelligent bucket Integration with a 0.04 ppm bucket width using ACD NMR Processor (ACD Labs Inc, Toronto, Canada). Integrals of each of the bins were normalized to total integral of each of the spectrum.