Title: Identification and Quantification of Metabolites in a Human Plasma Standard Reference Material by Multiple Mass Spectrometry Methods

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Novel Aspect: This is the first metabolomics standard reference material from a national metrology institute.

Introduction: Metabolomics attempts to understand biological function or disease through broad surveys of metabolites in relevant samples. Experiments are either identification based or quantification based; or a combination of both approaches is used. Standard reference materials (SRMs) establish traceability to a common sample, thus benchmarking the analytical method and/or improving the quantitative accuracy by providing a point of reference for calibration procedures. The intended use for NIST SRM 1950 is the evaluation of qualitative and quantitative analytical methods for metabolites in human plasma. The concentrations of approximately 40 metabolites were determined; most with isotope-dilution mass spectrometry. An additional approximately 250 metabolites were identified in the plasma using GC/MS and LC/MS instrumentation and mass spectral library searching.

Methods: SRM 1950 is a human plasma pool designed to represent a normal metabolome. It was obtained from an equal number of men and women in a narrow age range, with a racial distribution similar to that of the U.S. population. Contributing individuals should not have taken medication prior to the blood draw and were free from overt diseases, and disorders. The approximately 40 metabolites were quantified using established definitive methods developed at NIST. Metabolite identification was performed primarily using 2 instruments: GCxGC/TOF and LC/ion trap MS, with GC/quadrupole MS, LC/Q-TOF and NMR used to verify identifications. The data was managed using a custom database and the R statistical programming language. The metabolites were classified according the uncertainty in their identifications.

Preliminary Data: The metabolites were quantified using several methods, each optimized specifically for a group of closely related target analytes. Fatty acids, glucose, and cholesterol were quantified using isotope-dilution GC/MS methods. Testosterone, cortisol, progesterone, folates, homocysteine, creatinine, and amino acids were quantified using isotope-dilution LC/MS/MS methods. Carotenoids, retinol, and tocopherols measured by LC-UV. Total protein was determined using UV-VIS spectrophotometry. The concentration of the metabolites in SRM 1950 ranged from approximately 0.001 pmol/µL to 10000 pmol/ µL. The approximately 250 identified metabolites were amino acids, organic acids including fatty acids, sugars, pharmaceuticals (contrary to the plasma pool requirements) and other miscellaneous compounds. Metabolites were identified by searching the NIST electron impact or MS/MS mass spectral libraries. In some cases, authentic reference compounds were used for verification. The ability of the different mass spectrometers to identify various types of metabolites was compared. To meet reporting guidelines and assist with the evaluation of qualitative methods, NIST will

make the information it used to identify the metabolites publically available. This includes, for both the identified metabolites and the authentic reference compounds, a detailed description of the methods, retention times/retention indices, tandem mass spectra, electron impact mass spectra, and total ion chromatograms obtained on various chromatographic stationary phases. If the user's quantitative method is for a metabolite(s) with a certified concentration in SRM 1950, the SRM can be used to assess the method's accuracy with traceability to NIST. SRM 1950 can also be used to evaluate profiling or identification methods by benchmarking the ability of the method to identify specific metabolites or a certain number of metabolites in plasma. This metric can be tracked over time to check method repeatability, or as the method is intentionally changed.