**RTI-RCMRC-Metallomics-Analysis**

**ICP-MS Methods**

Prior to digestion, all samples were gently vortexed to provide a homogeneous matrix for digestion. Samples were immediately pipetted to prevent settling prior to removing the sample. A volume of 150 µL of each serum sample was dispensed into an acid-washed plastic digestion tube. To each digestion vessel, 300 µL of concentrated HNO3, 150 µL of concentrated HCl, and 100 µL of H2O2 solution was added. Samples were loosely capped and placed in the graphite digestion block and heated at a temperature of 90 °C for 2 hours. At the end of digestion, all samples were removed from the heating block and allowed to cool to room temperature. In the clean hood, samples were spiked with a multielement internal standard to provide a final concentration of 1.0 ng/mL Sc, In, and Pr, and diluted to the final volume with deionized (DI) H2O. Samples were stored in a monitored refrigerator at a nominal temperature of 8 °C until analysis.

**Standard and QC Preparation**

As many of the analytes are present at endogenous concentrations in serum, solvent calibration standards were prepared using NIST-traceable 10 mg/L elemental standards. Reagent blanks were prepared by addition of deionized water in place of serum. For each analytical day, seven reagent blanks were prepared to monitor background concentrations of all analytes. In addition, pooled serum samples were prepared with each analytical batch and were compared to indicate reproducibility between analytical days. Please refer to 2. Metals and ESA Response Study Design Table.xlsx column “Raw Data Folder and Date of Analysis” for information regarding which samples were analyzed in each of the four analytical batches.

**ICP-MS Method Parameters**

|  |  |
| --- | --- |
| **Mass Spectrometer** | Thermo Element 2 Sector Field Inductively-coupled Plasma Spectrometer |
| **Nebulizer** | Glass concentric |
| **Injector** | 1.8 mm, quartz |
| **Cones** | **Ni** |
| **Spray Chamber** | Glass cyclonic, Peltier-cooled |
| **Internal Standards** | Scandium (Sc), indium (In), and praseodymium (Pr) |

**ICP-MS Parameters**

Resolution Mode Analyte Dwell(ms) Accurate Mass

Medium 27Al 10 **26.9810**

Medium 51V 10 **50.9434**

Medium 52Cr 10 **51.9400**

Medium 55Mn 10 **54.9375**

Medium 59Co 10 **58.9327**

Medium 58Ni 10 **57.9348**

Medium 60Ni 10 **59.9302**

Medium 63Cu 10 **62.9291**

Medium 65Cu 10 **64.9273**

Medium 64Zn 10 **63.9286**

Medium 66Zn 10 **65.9255**

Medium 95Mo 10 **94.9053**

Medium 96Mo 10 **95.9041**

Medium 98Mo 10 **97.9049**

Medium 110Cd 10 **109.9025**

Medium 112Cd 10 **111.9022**

Medium 114Cd 10 **113.9028**

Medium 118Sn 10 **117.9011**

Medium 120Sn 10 **119.9017**

Medium 121Sb 10 **120.9033**

Medium 206Pb 10 **205.9739**

Medium 207Pb 10 **206.9753**

Medium 208Pb 10 **207.9761**

High 75As 10 **74.9211**

High 78Se 10 **77.9168**

High 82Se 10 **81.9162**

**Typical MS Parameters**

Plasma Power(W): 1333

Extraction (V): -2000

Sample Gas (L/min): 1.175

Cool Gas (L/min): 16.50

Aux Gas (L/min): 0.90