1. **MATERIALS**
   1. **CHEMICALS AND REAGENTS**

* + - * + NaH2PO4, Sodiumphosphatemonobasic anhydrous (Fisher Scientific)
        + Na2HPO4, Sodiumphosphatedibasic (Fisher Scientific)
        + HCl, Hydrochloric acid (Fisher Scientific)
        + NaOH, Sodium hydroxide (Fisher Scientific)
        + D2O, Deuterium oxide, 99.9 atom % D (Cambridge Isotope Laboratories, Inc.)
        + DSS-D6, Sodium 2,2-dimethyl-2-silapentane-5-sulfonate, 98 atom % D (Cambridge Isotope Laboratories, Inc.)
        + Pooled quality control (QC) Normal Human Red Cross plasma
  1. **Equipments**
     + - * Calibrated micropipettes (100 μl, 200 μl, and 1000 μl)
         * Pippette tips
         * 1.5 ml eppendorf tubes
         * 5 mm SampleJet NMR tubes from Bruker
         * 50ml volumetric flask
         * Eppendorf centrifuge
         * Analytical Weighing balance
         * Vortex Genie 2
         * pH meter
         * Labels
         * Speed-vac concentrator

1. **PROCEDURE**
   1. **PHOSPHATE BUFFER PREPARATION (**0.075 M Na2HPO4 buffer)
      * + - Dissolve 0.532 g of Na2HPO4 in 30ml of water
          - Add 1ml of 0.1 M DSS-D6 stock solution and vortex it until the powder is dissolved
          - Add 1.5 ml of 0.1 M NaN3 aqueous solution and mix it thoroughly
          - Add 10 ml of D2O
          - Adjust the pH to 7.4
          - Transfer the mixture to a 50 ml volumetric flask and adjust the volume to 50 by water and mix it well
          - Recheck the pH
          - Store buffer at 4°C
   2. **GENERAL GUIDELINES** 
      * + - Frozen samples should be thawed in 4ºC cold room or on ice
          - Check for the minimum volume of all the samples received
          - All sample preparation has to be done at 4ºC cold room or on ice
          - For 48 samples or less use two blanks (NMR buffer containing DSS), one at the beginning and one at the end
          - For more then 48 samples use three blanks, at the beginning, middle, and the end of the set
          - Total number of QC samples is 10 % of study samples. One QC after the first blank at the beginning and one before the last blank at the end of the run. The rest of the QC samples are randomized with the client samples using excel randomizer
   3. **SAMPLE PREPARATION FOR NMR**
2. Thaw samples at 4°C cold room or on ice
3. Mix 300 µl of phosphate buffer with 300 µl of sample in a 1.5 ml eppendorf tube.
4. Vortex each sample for 2 min
5. Centrifuge at 16,000 rcf for 15 min at 4°C.
6. Transfer 580 µl of supernatant into 5 mm SampleJet NMR tube.
7. Keep NMR samples on ice and transfer to NMR instrument to acquire data, if there is any short delay keep them in 4°C refrigerator
8. **REFERENCES:**
9. Anthony, C., *et al*. Precision High-Throughput Proton NMR Spectroscopy of Human Urine, Serum, and Plasma for Large-Scale Metabolic Phenotyping. Analytical Chemistry 2014, **86 (19)**, 9887-9894.

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