## Standard Operating Procedure-Plasma/Serum for GC-MS

### *1. Purpose*

To extract metabolites from serum/plasma for general metabolomics analysis by GC-MS. This procedure covers processing the actual samples, preparation of process blanks (PB) samples to determine sources of possible contamination, and quality control (QC) samples.

### *2. Scope*

This SOP applies to all serum/plasma samples submitted for GC-MS analysis. Samples may come from academic laboratories or outside companies.

### *3. Prerequisites*

Agreement between the client and our lab.

### *4. Responsibilities*

Dr. James Cox is the primary researcher responsible for this SOP and the assays involved herein. Dr. Alan Maschek and Steven Bell are also covered in this SOP.

### *5. Procedure*

**Serum/plasma sample preparation-Researcher**

a) Sample Preparation. Samples (serum or plasma) should be prepared, aliquoted into 40 µL fractions using 1.5 mL microcentrifuge tubes, snap frozen and kept at -80°C. It is important when preparing plasma to use $K_3$EDTA tubes.
b) Submit samples to Core with a hard copy of sample submission form. This will be placed into sample box for identification.

**Serum/plasma sample extraction-Core**

c) Determine quantity of 90% methanol (MeOH) solution needed for extraction. For each sample 9 parts of 90% HPLC grade methanol is added to each part serum/plasma. **Example:** If one has 12 samples of 40 µL of serum then 12 X 360 µL 90% MeOH= 4320 µL is needed. Include 2 extra samples for process blanks. For accurate measurement assume that you will need enough for 15 samples, 15 X 360 µL= 5400 µL or 5.4 mL. Make this up in a 15 mL or 50 mL conical tube. ***Use 10 mL pipette.***
d) Determine quantity of d4-succinate internal standard to add from stock solution. Total amount of d4-succinate per sample is always 1 µg. The stock

solution is made of 1 mg/mL of d4-succinate made up in nanopure water. Add to 90% methanol solution and chill to -20°C in freezer. ***Use positive displacement tip for this.*** **Example:** For methanol prepared in c) add 1 µL of stock solution of d4-succinate.

e) Determine quantity of d27-myristic acid internal standard to add from stock solution. Total amount of d27-myristic acid per sample is always 15 µg. Add to 90% methanol solution and chill to -20°C in freezer. ***Use positive displacement tip for this.*** **Example:** For methanol prepared in c) add 15 µL of stock solution of d27-myristic acid.

f) Thaw samples on ice or in a 4°C refrigerator prior to extraction. Start this after solutions are prepared. Label 1.5 mL microfuge tubes as PB (Process Blank). For 12 samples make up 2, for 18 make up 3, for 24 make up 4, etc.

***g)*** Using **piston pipette** add the cold 90% methanol solution containing the internal standard into each tube including the PB. ***It is important to use the piston pipette for accurate addition. Make certain no bubbles are in the tip!***

h) Vortex for 30 seconds each sample, place into -20°C freezer and incubate for 1 hour. ***Use timer!***

i) Chill Beckman centrifuge and rotor to 4°C during this time. Label a fresh set of 1.5 mL centrifuge tubes with the exact label as found on the sample tubes.

j) After incubation in step h) centrifuge at 20,000 x g for 5 minutes at 4°C.

k) Pour supernatant from each sample into the labeled tubes.

l) Make up QC samples by removing 20% volume of each sample and adding to a single micro centrifuge tube. From this aliquot an amount equal to the final volume of the actual samples. **Example**: For the 12 x 40 µL of serum used in step c) remove 80 µL from the samples in j). This gives a final volume of each sample of 320 µL. Mix the pooled QC by vortex then aliquot 320 µL into 3 tubes labeled QC. *THIS STEP IS CRITICAL-DO NOT SKIP!*

m) Dry samples in speed-vac using *–OH* setting overnight. It is critical that extracted samples are completely dry.

n) Store at room temperature in box containing several desiccant bags.

**GC-MS sample preparation**

o) Prepare GC-MS using operating SOP

p) Make up a 40 mg/mL solution of N-methoxy amine (MOX) in dry pyridine. The MOX is stored in room temperature desiccators. The dry pyridine is stored in the hood. Dry the needle and syringe prior to use

**Waste disposal**

a) Chemical waste generated from sample extraction and LC-MS analysis are collected and pooled for collection from University Utah Environmental Health and Safety.

## *6. References*

## *7. Definitions*

GC: gas chromatography
MS: mass spectrometry
PB: process blank
QC: quality control