Stedman Metabolomics Laboratory notebook CN196: 012.

Goal: Prepare **human plasma** for non-targeted metabolomics via gas chromatography/mass spectrometry (GC/MS). **One "ghost" process blank will also be prepped**.

Thaw and vortex the samples.

Number pre-cleaned 1.7-ml polypropylene microcentrifuge tubes. These will have a black tick mark on the underside of the hinge.

Make 100-µl aliquots of the samples. Record the volume aliquotted if it comes up short of 100 µl, and scale subsequent reagent volumes accordingly.

Crash precipitate proteins with 750 µl methanol spiked with C14:0-D_{27} (6.25 mg/liter, CN188: 115). Vortex. Spin hard for 10 minutes.

Transfer 700 µl of supernatant to fresh, pre-cleaned tubes.

SpeedVac until just dry, typically about 4.5 to 6 hours.

Add 100 µl ethyl acetate to each tube. Vortex. SpeedVac for a half hour to assure thorough azeotropic drying. Close tubes and store them at -80°C for up to one week, if necessary.

Add 25 µl methoxyamine hydrochloride (18 mg/ml in dry pyridine, CN195: 174). Vortex. Pulse spin to push liquids to the bottom of the tube.

Transfer to <u>pre-warmed</u> tube rack. Incubate at 50°C for 30 minutes for methoximation of reactive carbonyl groups.

Add 75 µl MSTFA (*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide, Cerilliant M-132, Sigma, St. Louis, MO) to trimethylsilyl replacement of exchangeable protons. Vortex. Pulse spin to push liquids to bottom of tube and to sediment any precipitate.

Transfer to <u>pre-warmed</u> tube rack. Incubate at 50°C for 30 minutes for trimethylsilylation.

Transfer to glass GC vial for GC/MS analysis.