Issued: 02-25-2019 Valid from: 02-27-2019	Validity area: UC Davis Genome Center, Metabolomics Core and Research Laboratories
Responsible: Oliver Fiehn	Secondary: Luis Valdiviez
This SOP supersedes: extraction lipidomics	Approved: Oliver Fiehn

Sample preparation of blood plasma or serum samples for lipidomic analysis

1. Purpose:

This SOP describes sample extraction and preparation of blood plasma or serum for lipid profiling on the CSH platform by liquid chromatography/quadrupole time-of-flight mass spectrometry (LC-QTOF).

2. References:

Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A and Schwudke D (2008) Lipid extraction by methyl-*tert*-butyl ether for high-throughput lipidomics. J Lip Res 2008, 49: 1137-1146

3. Starting material:

Plasma/serum: 20 µL sample volume or aliquot

4. Equipment:

- Centrifuge Eppendorf 5415 D
- Calibrated pipettes 20-200µL and 100-1000µL
- Multi-Tube Vortexer (VWR VX-2500)
- Orbital Mixing Chilling/Heating Plate (Torrey Pines Scientific Instruments)
- Speed vacuum concentration system (Labconco Centrivap cold trap)

5. Chemicals:

Product	Manufacturer & Part Number
Eppendorf tubes 1.5 mL, uncolored	Eppendorf 022363204
Crushed ice	UC Davis
Water, LC/MS Grade	Fisher Optima W6-4
MTBE, HPLC Grade	Acros Organics 389050010
Methanol, LC/MS Grade	Fisher A456-4
Bioreclamation human plasma (disodium EDTA)	Bioreclamation HMPLEDTA

6. Sample Preparation:

Preparation of extraction solvent

Combine 120 mL of chilled MeOH/QC mix with 400 mL of chilled MTBE/Cholesterol Ester 22:1 in a clean 500 mL stock bottle. Mix thoroughly by swirling or stir plate and store at -20°C until use.

*See SOP "QC mix for LC-MS lipid analysis" for preparation of MeOH/QC mix and MTBE/Cholesterol Ester 22:1.

Extraction

1. Thaw raw samples/controls at room temperature (or in the refrigerator at 4˚C) and either invert the tube or vortex 10 sec at **low speed** to homogenize.
2. Aliquot 20 µL of plasma sample into a 1.5 mL Eppendorf tube. Keep all samples on ice.
3. Add 975 µL ice-cold 3:10 (v/v) MeOH/MTBE + QC mix/CE 22:1 extraction solvent mixture to each

aliquot, keeping the extraction solvent on ice during the procedure.

4. Vortex samples for 10 seconds, then shake for 5 minutes at 4°C on the orbital mixer.
5. Add 188 µL room temperature LC/MS grade water to each tube.
6. Vortex tubes for 20 seconds and then centrifuge for 2 min at 14,000 rcf.
7. Transfer the upper organic phase to two separate tubes (350 µL/each tube) for lipidomics analysis.
8. Transfer 75 µL of the remaining organic phase to a 2, 15, or 50 mL tube for pools, depending on number of samples in the study.
9. Transfer the bottom aqueous phase to two separate tubes (110 µL/each tube) for HILIC/GC-TOF analysis.
10. Dry down one tube from each phase by centrivap, keeping the undried tubes as backups. Store all tubes at -20˚C until ready for analysis.
11. Submit to resuspension.

Pooling (CSH platform only)

1. Transfer multiple 350 µL aliquots of pooled samples to 1.5 mL Eppendorf tubes, one aliquot for every 10 samples in the study. If there is still pool remaining, prepare additional aliquots for backup.
2. Evaporate to complete dryness in the Labconco Centrivap cold trap concentrator. Store all tubes at -20°C until ready for analysis.

7. Quality assurance

- For every 10 samples, extract a method blank (20 µL of H_2O) and a sample control (20 µL human Bioreclamation or analogous species plasma) in addition to samples.
- For large studies (>100 samples), for every 100 samples a NIST plasma extract should be prepared in the same manner as positive controls.

8. Disposal of waste

- Collect all chemicals in appropriate bottles and follow the disposal rules.
- Collect residual plasma/serum samples in specifically designed red ‘biohazard’ waste bags.

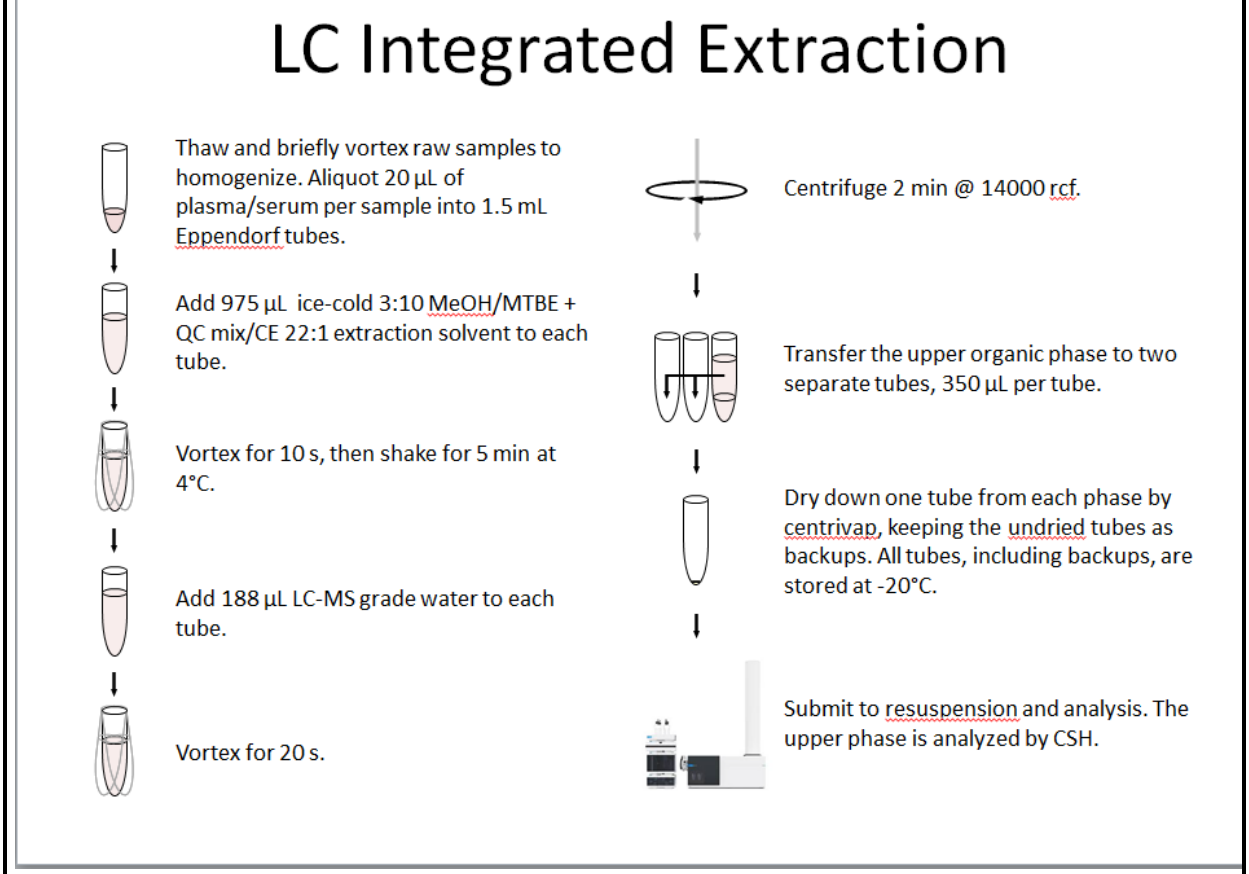

Figure 1. Integrated Extraction Procedure Overview