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Responsible:	
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Extraction of Blood Plasma/Serum

1. References:

- Fiehn O, Kind T (2006) Metabolite profiling in blood plasma. In: Metabolomics: Methods and Protocols. Weckwerth W (ed.), Humana Press, Totowa NJ (in press)

2.Starting material:

- Blood plasma/serum: 5-30ul sample volume (or aliquot) (validated for plasma)
- Other body liquids: 5-30ul sample volume (or aliquot) such as liquor, urine (not validated)

3. Equipment:

- Centrifuge (Eppendorf 5415 D)
- Calibrated pipettes 1-200µl and 100-1000µl
- Eppendorf tubes 2ml, uncoloured (Cat.No. 022363204)
- ThermoElectron Neslab RTE 740 cooling bath at –20°C
- MiniVortexer (VWR)
- Orbital Mixing Chilling/Heating Plate (Torrey Pines Scientific Instruments)
- Speed vacuum concentration system (Labconco Centrivap cold trap)

4. Chemicals

- Acetonitrile, LCMS grade (JT Baker; Cat. No.9829-02)
- Isopropanol, HPLC (JT Baker; Cat. No. 9095-02)
- Crushed ice
- pH paper 5-10 (EMD Chem. Inc.)
- Nitrogen line with pipette tip
- 18 MΩ pure water (Millipore)

5. Preparation of extraction mix and material before experiment:

1. Switch on bath to pre-cool at –20°C (±2°C validity temperature range)
2. Check pH of acetonitrile and isopropanol (pH7) using wetted pH paper
3. Make the extraction solution by mixing acetonitrile, isopropanol and water in proportions 3 : 3 : 2
4. Rinse the extraction solution mix for 5 min with nitrogen. Make sure that the nitrogen line was flushed out of air before using it for degassing the extraction solvent solution

6. Sample preparation:

1. Vortex the plasma/serum samples for 10s to obtain a homogenized sample using the MiniVortexer.
2. Aliquot 15-30ul and add 1mL extraction solution. The extraction solution has to be pre-chilled using the ThermoElectron Neslab RTE 740 cooling bath set to -20°C.
3. Vortex the sample for 10s and shake for 5min at 4°C using the Orbital Mixing Chilling/Heating plate. If you are using more than one sample, keep the rest of the samples on ice (chilled at <0°C with NaCl).
4. Centrifuge samples for 2min at 14000 rcf using the centrifuge Eppendorf 5415 D.
5. Aliquot two 500µL portions of the supernatant. One for analysis and one for backup. Store one aliquot in the -20°C freezer as a backup.
6. Evaporate one 500µL aliquot of the sample in the Labconco Centrivap cold trap concentrator to complete dryness.

The following steps are **not** done with urine:

7. The dried aliquot is then re-suspended with 500µL 50% acetonitrile (degassed as given above).
8. Vortex the samples for 10s using the MiniVortexer VWR.
9. Centrifuge for 2min at 14000 rcf using the centrifuge Eppendorf 5415 D.
10. Remove supernatant to a new Eppendorf tube.
11. Evaporate the supernatant to dryness in the Labconco Centrivap cold trap concentrator.
12. Submit to derivatization.

The residue should contain membrane lipids because these are supposedly not soluble enough to be found in the 50% acetonitrile solution. Therefore, this 'membrane residue' is now taken for membrane lipidomic fingerprinting using the nanomate LTQ ion trap mass spectrometer. Likely, a good solvent to redissolve the membrane lipids is e.g. 75% isopropanol (degassed as given above). If the 'analysis' aliquot is to be used for semi lipophilic compounds such as tyrosine pathway intermediates (incl. dopamine, serotonine etc, i.e. polar aromatic compounds), then these are supposedly to be found together with the 'GCTOF' aliquot. We can assume that this mixture is still too complex for Agilent chipLCMS. Therefore, in order to develop and validate target analysis for such aromatic compounds, we should use some sort of Solid Phase purification. We re-suspend the dried 'GCTOF' aliquot in 300 □l water (degassed as before) to take out sugars, aliphatic amino acids, hydroxyl acids and similar logP compounds. The residue should contain our target aromatics .We could also try to adjust the pH by using low concentration acetate or phosphate buffer. The residue could then be taken up in 50% acetonitrile and used for GCTOF and Agilent chipMS experiments. The other aliquot should be checked how much of our target compounds would actually be found in the 'sugar' fraction.

6. Problems

To prevent contamination disposable material is used. Control pH from extraction mix.

7. Quality assurance

For each sequence of sample extractions, perform one blank negative control extraction by applying the total procedure (i.e. all materials and plastic ware) without biological sample.

8. Disposal of waste

Collect all chemicals in appropriate bottles and follow the disposal rules.