

SOP Title: Plasma and urine collection and processing for metabolomics fingerprinting and profiling in humans

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Research area: Metabolomics

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We used the NuGO Standard Operating Procedure (SOP) number 80 produced by Newcastle University. Details of the SOP are available via the web link: <http://www.nugo.org/frames.asp?actionID=39144&action=loginFromPP>

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Background: Plasma and urine metabolite profiles in humans are very sensitive to numerous and various factors ranging from water consumption or physical activity prior to sample collection, to the type of consumable used for sampling. These must therefore be measured in very carefully collected, processed and stored samples. The following standard operating procedure allows reproducible determination of plasma and urine metabolite profiles in humans by using either FIE-MS or GC-MS.

Subjects' preparation

On the day before sampling, subjects should be provided with a standardised evening meal, to be the same one for all subjects, and not containing any food susceptible to affect significantly their metabolome, such as whole grain or polyphenol-rich foods. Volunteers should also be specified to avoid any important physical activity and not to consume any alcohol. Their water consumption should be standardised and recorded to adjust, if necessary, their metabolomics profiles.

Consumable

All consumable should be purchased as batches.

Product	Reference	Details	Brand
4 mL Lithium/Heparin vacutainer [®] tube	367883	Plastic, LH 68 I.U.	Becton Dickinson
1.5 mL Micro tube	72.690	Polypropylene, safety cap	Sarstedt
Jug with spout and moulded graduations PP 500 mL	FB55940	Polypropylene	Fisherbrand
24h urine container	UR2401	Polyethylene	ISS Ltd
50 mL Falcon [®] tube	352070	Polypropylene, flat seal screw	Becton Dickinson

Plasma collection, processing and storage

Samples definition

Plasma samples should be collected using vacutainer[®] tubes coated with Lithium/Heparin to prevent clotting. Vacutainer[®] tubes coated with EDTA should not be used for metabolomics analysis, as EDTA dramatically affects the mass spectrometry chromatograms. Plasma will be obtained from non-clotted whole blood by spinning the tube until the blood cells fall to the bottom of the tube.

Samples collection, processing and storage

The vacutainer[®] tubes should not be shaken whilst handling, and the time of collection should be recorded. Each tube should be filled up as much as possible (to insure a reproducible concentration of preservative in the samples), inverted gently 3 times after collection, then placed horizontally on ice (not in ice) to prevent red cell lysis and to reduce protease activity.

If other tubes are collected, for example to characterise subject's nutritional status (measurement of serum glucose, lipids, etc...), the tubes intended for metabolomics measurement should be collected first, to prevent potential lysed cells stocked in the needle to contaminate the sample.

After collection, spin the vacutainer[®] tubes at 1,200 g for 10 minutes at 4°C, then, using a transfer pipette, decant the plasma (top layer) as aliquots into Micro tubes pre-labelled according to their destination, and store these straight forward at -80°C.

Urine collection, processing and storage

Samples definition and collection

Two different types of urine samples are worth being collected: spot samples and 12h samples.

The spot urine samples should be collected into jugs tested for not releasing any plasticiser of any kind into the sample (the time of collection should be recorded), after a 12h minimum fast for the fasting spot samples, or on several time points up to 4h minimum after a treatment. If subjects need to go to the toilet before time point of the next urine collection, they should collect this void into their jug and transfer it into a 2.5L urine container to be kept on ice until collection of the following void later at the collection time point. Both voids should then be pooled as one sample before processing, and time of collection of the 'early' sample should be recorded too.

The 12h urine sample intends to reflect the metabolism of the subject after consumption of the standardized evening meal on the day before the spot samples collection. The subjects should be asked to empty their bladder before having their standardised evening meal and to collect their urine from the end of their meal to their first void when getting up from bed on the following morning (included). Subjects should be provided with a suitable jug into which to urinate and with a urine container to transfer their urine into. Subject should be specified to record their times of urine collection, and not to wash or even rinse their jug at all. No preservative should be added into the container and no chill bag is required to store their container.

Samples processing and storage

After measurement of the volume of the whole urine samples, decant in the required number of pre-labelled Micro tubes and Falcon[®] tubes, discard the rest of the sample, and store all aliquots immediately at -80°C. No centrifugation is required before freezing but should be done after defrosting the samples, as a step in the preparation for mass spectrometry analysis.

If several urine samples are collected from any one subject, re-use their allocated jug, which should only be rinsed with tap water then distilled water and placed on tissues on the bench to dry.

