Appendix C

Singapore Phenome Centre
SOP (Collection of human clinical samples: urine, plasma, serum)

Urine is the most convenient, non-invasive[1] biological fluid for metabolite profiling. Careful collection of urine mandates the useful data collection. If untimely, collected the data obtained may show discrepancies in the metabolic profiling which will void the analysis. Hence, pre-defined time points with a proposed hypothesis should be ascertained during urine collection for metabolomics studies.

Plasma or serum contributes 55 % of the blood in human body. These biofluids contain many hundreds of metabolites[2] and hence provide an appropriate overview of different metabolic routes in the human body and represent the metabolic foot print of tissue metabolism. Analysis of plasma or serum in human subjects represents the phenotypes of different parts of the body in a single sample. Hence, in metabolomics studies these biofluids are of utmost importance to explore and identify the novel biomarkers or representative differences in the healthy versus disease state.

Material and Methods:

Material

Polypropylene tubes, vacutainers, eppendorfs, pipettes, cold centrifuge, tips, -20^{o}C refrigerator, 4^{o}C refrigerator, sample storage racks, sterile syringe, sterile, needles, urine sample collectors, cotton,

Chemicals

Sodium azide, lithium heparin, 70 %ethanol

Procedure:

Collection of Urine:

Urine can be collected at both timed and 24 h collections. The subjects should be given a urine container and asked to collect the mid-stream urine of approximately 10 mL. For timed samples, the collected urine samples should be sub-aliquoted to a volume of 1mL in eppendorf tubes and stored at -20^{o}C or lower temperatures. For 24 h sample collection, the collected sample should be stored at 4^{o}C between the collections and sub-aliquoted to a volume of 1 mL into the eppendorf tubes after 24 h collection and stored at -20^{o}C or lower temperatures. To avoid microbial contamination, the urine

sample should be added with 0.1% (w/v) sodium azide before storage. Sub-aliquoting the urine samples is to avoid repeated freeze thaw cycles which may cause depletion of metabolites.

Collection of Plasma or serum:

Draw blood (10 mL) from the peripheral vein using aesthetic procedures and transfer 5mL to a vacutainer with no anticoagulant and 5 mL to vacutainer containing lithium heparin. Allow the vacutainers to stand at room temperature for 15-20 min, the serum gets separated. Avoid any disturbances during serum separation. Vacutainers containing blood and lithium heparin should be centrifuges at 2000-3000 rpm at 4^{o}C for 10 min to separate the plasma. The separated serum and plasma should be sub-aliquoted (500 uL) in to eppendorf tubes and stored at -20^{o}C or lower temperature until sample preparation. The sub-aliquoting prevents repeated freeze thaw cycles which may cause metabolite depletion.

References:

1. Global metabolic profiling procedures for urine using UPLC-MS. Elizabeth J Want et al., Nature Protocols, 2010, 5(6), 1005-1018.
2. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Warwick B Dunn et al., Nature Protocols, 2011, 6(7), 1060-1083.

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