Sample Collection from Healthy/Ambulatory Control Subjects

SOP for whole blood collection for NMR metabolomics

On the day of collection, subjects should be instructed to refrain from exercise and to be well hydrated. Collection should be planned for between 0830-0930 and the patient should be fasting (@ least 12 h).

The sample should be collected by direct venipuncture if possible. If it is collected from an indwelling line then there should be at least a 5mL discard regardless of whether the line is "heparin locked."

Samples for plasma and whole blood need to be collected into Becton-Dickson (BD) green top tubes.[1] Each tube should be filled until the vacuum is exhausted and blood flow ceases.

Following collection, completely and gently invert the tube five times and immediately place in an ice-water bath.

For whole blood samples: Within 15 min of collection, keep blood on ice and generate up to five samples/patient by aliquoting 1mL of whole blood into each of five sterile (autoclaved) microfuge tubes (1.5mL). Place tubes on ice for 10 min then freeze (-80°C).

SOP for serum collection and processing for NMR metabolomics

On the day of collection, subjects should be instructed to refrain from exercise and to be well hydrated. Collection should be planned for between 0830-0930 and the patient should be fasting (@ least 12 h).

The sample should be collected by direct venipuncture if possible. If it is collected from an indwelling line then there should be at least a 5mL discard regardless of whether the line is "heparin locked."

Samples for serum will be collected into a 5 mL Becton-Dickson (BD) serum separator tube (SST).[2] The tube should be filled until the vacuum is exhausted and blood flow ceases.

Following collection, completely invert the SST tube five times to facilitate clotting.[3] Allow the blood to clot by placing the tube upright in a test tube rack at room temperature for 30 min. Observe a dense clot. Incomplete clotting can influence serum acquisition but this time should not exceed 2 h.

After the specimen has clotted, acquire serum by centrifugation. Samples should be centrifuged (1000 x *g*) in a refrigerated centrifuge with the temperature set at 25°C for 10 min in swing-bucket or 15 min in fixed-angle bucket centrifuges, respectively.[4]

Immediately following centrifugation, harvest serum and aliquot at least three (1mL each) into three sterile (autoclaved) microfuge tubes (1.5mL). Place tubes on ice for 10 min then freeze (-80°C).

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[1] For example, 16 x 100 mm x 10.0 mL BD Vacutainer® glass (plastic tubes should NOT be used) tube with a green conventional or Hemogard™ closure. Additive: Sodium heparin^{N} (freeze-dried) only- use of lithium heparin should be avoided. A number of BD green top tubes are acceptable: 366387, 366480, 367671, 367673, 367674, 367676, 367869

[2] 13 x 100 mm x 5.0 mL BD Vacutainer® SST II Plus plastic serum tube. Gold BD Hemogard™ closure. Paper label. CE. Additive: Clot activator and gel for serum separation. Silicone coated interior.

[3] http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_November2005_VS7436.pdf

[4] for a horizontal (swing-bucket) centrifuge, the recommended spin time is 10 min; for a fixed-angle centrifuge, the recommended spin time is 15 min