BILIARY MALIGNANCY PROTEOMICS AND METABONOMICS STUDY

Sample Collection SOP B: for participants undergoing ERCP or PTC procedures

SAMPLE COLLECTION

Confirm suitability, issue PIS, obtain **informed consent**. Copy of consent form to patient and notes

Complete CRF including demographic details, clinical details, dietary and drug history

A total of 4 tubes and **16 mLs** of blood is required. Note the time of blood collection on the CRF

Take one **4mLs EDTA** tube (PURPLE TOP VACUTAINER) for proteomic and genomic studies

Take one **4mLs plain serum/no-additive** tube (BRIGHT RED TOP VACUTAINER) for metabonomic studies using MR spectroscopy

Take one **4 mls lithium heparin** tube (GREEN TOP VACUTAINER) for metabonomic studies using MR spectroscopy

Take one **4mls SST** tube (YELLOW TOP VACUTAINER) for standard biochemical analysis

Obtain **≥ 15mLs** urine in plain, additive free universal container

Obtain **1-20 mL** of contrast free bile at time of ERCP/PTC into sterile, plain specimen tube. Wrap in foil to protect from light. If it is impossible to obtain contrast free bile, contrast contaminated bile should be collected. Metabonomic studies will **not** be possible on contaminated bile.

All samples (blood, bile and urine) should be kept on ice with light excluded whilst processing is awaited

Samples should be left for a minimum of 30 minutes before processing, but should be processed as soon as possible after this. Samples should be processed within a maximum of 2.5 hours. Collection and processing times should be documented on the CRF.

Include summary of ERCP/PTC findings on CRF.

SAMPLE PROCESSING

Blood.

Keep blood samples on ice or in +4 fridge whilst processing awaited
Spin all blood samples at +4 C at **1000g for 10 minutes**
Store 250 µL aliquots of each supernatant in Eppendorf tubes.
Please aim for a minimum of 4 aliquots from each vacutainer tube
Note the time of centrifuging on the CRF
Remove the buffy coat layer from the EDTA sample and store in a cryovial or Eppendorf tube
Discard all remaining cell layers in the vacutainers

Urine:

Keep urine on ice or in +4 C fridge while processing awaited
Spin the urine sample at +4 C at **1000g for 10 minutes**
Store 6 x2 ml aliquots of urine supernatant in Eppendorf tubes
Discard any remaining urine, cellular debris etc remaining in the urine tube

Bile:

Spin at 4°C at **16,000g for 10 minutes**. Store 200µL aliquots of supernatant in eppendorf tubes. Resuspend the pellet in half ml phosphate buffered saline. Agitate and mix. Spin at 4C **16000 g for 5 minutes**. Dispose of supernatant. Resuspend the pellet again in half ml phosphate buffered saline. Agitate and mix. Spin again at 4C **16000g for 5 minutes**. Again discard supernatant. Retain packed cells and debris in original tube.

All samples should be stored at -80°C as soon as possible. However, short term storage in a standard -20°C freezer is acceptable, pending transfer to a -80°C freezer

Labelling

A unique case/subject identifier should be allocated in the site master file
Each aliquot should be labelled with a code.
This code starts with the allocated case subject number (the first 3 letters of the site and 1,2,3 etc), followed by an alphabetic code for the type of sample, followed by the aliquot number.
The coding for sample type is as follows: **U** = urine, **S** = red cap serum, **E** = EDTA plasma, **H** = lithium heparin, **T** = SST serum, **BC** == buffy coat, **B** = bile
E.g. For the third aliquot of EDTA plasma from subject Sou5 (5th Southampton patient) the label would read **Sou5E3**

Record sample details in sample registry

Archive consent form and CRF securely.

Please direct any queries about this protocol to Mary Crossey
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