

APPENDIX G

GUIDELINES FOR SAMPLE COLLECTION -

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For all samples blood should be drawn:-

- a) Prior to any manipulation of the prostate
- b) At least 24h following ejaculation (if within 24h the time should be noted)
- c) 6 weeks after resolution of prostatitis

Each centre must record for each sample:-

- a) The tube used to collect the sample (should include full details of tube type and manufacturer)
- b) All sample manipulations eg
 - a. Time of blood draw
 - b. Time and temperature of centrifugation (where appropriate)
 - c. Time and temperature of storage

Details of the PSA test used:

- 1) Manufacturer (eg DPC, Roche, Bayer)
- 2) Kit (eg DPC IMMULITE Third Generation test)
- 3) Kit Lot number:

Samples to be collected:

Any sample marked (*) is optional. Centres may participate in as many of the optional studies as they wish. No sample collected should remain at room temperature or refrigerated +4°C for more than 24hours (ie one day)

- 1) **Sample collection for local PSA testing** (normally serum but some centres may be using tests that recommend plasma. There is no need to collect both in this section)

Volume: 5 - 7mL blood

For serum: Plain tube

For plasma: anticoagulant tubes as used by centre and recommended by manufacturer of the PSA tests being carried out by the centre's clinical laboratory

For serum:

Allow blood to clot at 4°C for between 30min and 90min.

Centrifuge at ~1900g for 15 minutes. (In a typical clinical centrifuge ~1900g is ~3400rpm. Please refer to manufacturer's tables for particular rotors and carriers)

Remove serum with a sterile pipette

*one aliquot of 0.5mL should be treated the same way as the bulk of the serum until PSA determination and then frozen at -20°C

The serum should be sent to the centre's clinical laboratory for routine PSA determination and any other tests requested by the GP/clinician

For plasma:

Sample should be processed as soon as possible after blood collection Centrifuge at ~1900g for 15 minutes. (In a typical clinical centrifuge ~1900g is ~3400rpm. Please refer to manufacturer's tables for particular rotors and carriers)

Remove plasma with a sterile pipette

*one aliquot of 0.5mL should treated the same way as the bulk of the plasma until PSA determination and then frozen at -20 °C

The plasma should be sent to the centre's clinical laboratory for routine PSA determination and any other tests requested by the GP/clinician

2) Serum for routine quality control

Volume: 5 - 7mL

Collection tubes: Plain

Aliquots: 2 x 0.5mL, 5 x 0.25 mL, as many 0.1mL aliquots as possible to end of sample. (1.8mL Nunc Cryotubes should be used to store the aliquoted samples)

Storage: The serum should be prepared as described above and transferred to a -20°C or colder (please make a note of the temperature). The samples may be stored at 4°C for up to 24 hours (if this presents a problem we can go to 48 hours)

Shipment: Samples should be shipped to The Institute of Cancer Research on dry ice once a quarter

3) Plasma for routine tests including PSA and hK2 and new markers in Prof. Lilja's lab

Volume: 5-7 mL

Collection tubes: Li-Heparin

Aliquots: as many aliquots x 0.5mL as possible to end of sample.

Storage: The plasma should be prepared as described above and transferred to a -20°C or colder (please make a note of the temperature). The samples may be stored at 4°C for up to 24 hours. Shipment: Samples should be shipped to The Institute of Cancer Research on dry ice once a quarter

4) *Serum for proteomic studies- it is important to treat all samples the same as regards processing time for this part

Volume: 5-7mL

Collection tube: Plain

Allow blood to clot at room temperature for exactly 60 mins.

Centrifuge samples for 15min at 4 °C

Place tube on ice. Remove serum with a sterile pipette and immediately dispense into 0.5mL aliquots on ice

Transfer the aliquots to a - 70 °C, or colder, freezer as soon as practically possible – ideally within 1 hour of centrifugation

Shipment: Samples should be shipped to The Institute of Cancer Research on dry ice once a quarter

5) ***Plasma for proteomic studies**

Volume: 5-7mL

Collection tube: Li-Heparin

Following collection place tube on ice

Centrifuge for 15min at 4 °C

Place tube on ice. Remove plasma with a sterile pipette and immediately dispense into 0.5mL aliquots on ice.

Transfer the aliquots to a - 70 °C freezer, or colder, as soon as practically possible – ideally within 1 hour of centrifugation

6) ***DNA for genomic studies**

Volume: ~ 6mL

Collection tube: EDTA

Tubes should be stored at -70 °C, or cooler. There is no need to centrifuge the tubes. Tubes can be left at -20 for up to two weeks before transfer to -70 °C

Shipment: Samples should be shipped to The Institute of Cancer Research on dry ice once a quarter

7) ***RNA for expression studies - Using the PAXgene™ Blood RNA tubes System**

Volume: 2 x 2.5mL

Collection tubes: PAXgene™ Blood RNA Tubes

Following blood collection in the PAXgene™ tube, the tube should be inverted 8-10 times and then stored upright. The samples should be left overnight at room temperature before freezing at -70°C.. As always the important thing is to keep processing as consistent as possible

The PAXgene™ Blood RNA Tubes should only be frozen in a wire rack, not in a polystyrene tray. The tubes should be frozen 18-24 hours after collection.

Shipment: Samples should be shipped to The Institute of Cancer Research on dry ice once a quarter

8) ***Extraction of Lymphocytes**

Volume: 10mL

Collection tube: Lithium Heparin

The blood can be kept at room temperature for up to 24 hours in the dark. Transfer to 50mL falcon tube and add 10mL PBS Dulbecco solution A. Underlay 10mL of lymphoprep under the blood and centrifuge for 30 minutes at 2000rpm (~500g, please refer to manufacturer's tables for particular rotors and carriers). Remove the top layer and buffy coat. Transfer to 50mL falcon tube and add equal volume of PBS Dulbecco solution A. Centrifuge for 10mins at 1200rpm (~250g, please refer to manufacturer's tables for particular rotors and carriers). Discard supernatant and tap to loosen pellet.

Storage: Add freezing mixture (2.7mL Foetal calf serum and 0.3ml (10%) DMSO per sample) to pellet and mix. Transfer 1.5mL to each freezing vial and Place in freezing container over night at -70°C and transfer to liquid nitrogen freezer.

9) *Urine collection

Volume: 1 x ~ 10mL collected in universal plastic containers.

Aliquot into 5 x 1.8ml Cryotubes

The urine should be frozen as soon as possible at -70 °C or colder. Do not overfill the plastic container as this will cause the tubes to crack on freezing due to volume expansion.

Shipment: Samples should be shipped to The Institute of Cancer Research on dry ice once a quarter

10) Urine for Proteomics

Centrifuge the urine sample at 1600g for 5 mins at 4°C

Place 600 µL urine into a 1.8mL cryotube

Add 900 µL of denaturing buffer * into the same tube

Aliquot 0.5 µL samples from this mix into 3 cryotubes.

Freeze at -80°C

Repeat with another 600 µL of urine and 900mL of buffer

To Make the Denaturing Buffer:

Requires: 9M urea and 2% CHAPS/ 50mM pH 9.0

For 10ml:

5.4g urea

0.2g CHAPS

Water to 10mls