1. INTRODUCTION

1.1. CADET collects biological samples and data from patients for use in biomedical research. All material is stored securely and in suitable tubes and containers.

2. SCOPE

2.1. This SOP defines the procedure for the collection of samples from patients and donors.

3. RESPONSIBILITIES

3.1. All CADET staff are responsible for ensuring they work to this SOP at all times.

3.2. Questions regarding technical aspects of this SOP can be directed towards Richard Unwin (r.unwin@manchester.ac.uk; 0161 701 0244)

4. HEALTH AND SAFETY

4.1. All human biological material is considered a biohazard and must be handled according to local Health and Safety rules.

4.2. Gloves and labcoat must be worn when handling any biological material.

5.1 Equipment

Equipment	Manufacturer	Serial Number
Bench-top refrigerated centrifuge	Any suitable	

5.2 Materials

Material	Manufacturer	Cat#
VACUETTE® 9ml serum collection tube	Greiner	455092
VACUETTE® 9ml lithium heparin plasma collection tube	Greiner	455084
VACUETTE® 6ml sodium heparin trace elements	Greiner	456080
Cryovials e.g. 2ml round bottom	Greiner	122261/122263

5.3 Chemicals

Chemical
Ice-cold saline solution (0.85% NaCl in water)
Ice-cold phosphate buffered saline
Ice-cold 0.25M sucrose

6. PROCEDURE

6.1. BLOOD COLLECTION GENERAL POINTS

6.1.1. Blood needs to be collected in the appropriate collection tube for serum and plasma. Blood must be packed well in wet ice after collection until it arrives at the laboratory for processing and must be stored on ice during processing in the laboratory. Ideally, sample transport and processing should be performed within 30 minutes. The involvement of research nurses or clinical fellows is advisable if possible. Avoid any exposure of samples to ambient temperatures after collection.

6.1.2. Use high quality plastics so that there is no leakage of plasticisers and contaminants into blood samples. We recommend the use of the materials listed in Table 5.2 as these have been applied for a number of years and we trust them regarding the absence of contamination.

6.2. SERUM COLLECTION (if collecting for metabolomics analysis only)

6.2.1. Draw blood (typically 9ml) into suitable serum collection tubes (Table 5.2)(DO NOT USE GEL-BASED CLOTTING TUBES) and allow to clot for a minimum of 1 hour at 0°C on ice. The clotting time (time from sample collection to centrifugation) must be recorded.

6.2.2. Prepare the serum fraction by centrifugation of the blood collection tube at 2500 x *g* for 15 minutes at 4°C.

6.2.3. Divide samples immediately into aliquots (0.5ml) in cryovials and freeze by placing in a -80°C freezer.

6.2.4. Collect enough blood (typically greater than 4ml) so that there is sufficient serum for at least 4 aliquots. Store samples at -80°C and transport on dry ice.

6.3. PLASMA COLLECTION (if collecting for proteomics and metabolomics studies)

6.3.1. Draw blood into Lithium heparin plasma collection tubes (Table 5.2) and mix it with the anticoagulant by inverting the tube three times. Prepare the plasma fraction immediately by centrifugation at 3,000 x *g* for 20 min at 4°C.

6.3.2. Divide samples immediately into aliquots (0.5ml) in cryovials and freeze by placing in a -80°C freezer.

6.3.3. Collect enough blood (typically greater than 4ml) so that there is sufficient plasma for at least 4 aliquots. Store samples at -80°C and transport on dry ice.

6.4. PLASMA COLLECTION (if collecting for trace metal analysis only)

6.4.1. Draw blood into sodium heparin plasma collection tubes (Table 5.2) and mix it with the anticoagulant by inverting the tube three times. Prepare the plasma fraction immediately by centrifugation at 3,000 x *g* for 20 min at 4°C.

6.4.2. Divide samples immediately into aliquots (0.5ml) in cryovials and freeze by placing in a -80°C freezer.

6.4.3. Collect enough blood (typically greater than 4ml) so that there is sufficient plasma for at least 4 aliquots. Store samples at -80°C and transport on dry ice.

7.1. TISSUE COLLECTION GENERAL POINTS

7.1.1. Tissue samples should be washed, frozen and stored as soon as possible after collection. Ideally wash solutions, liquid nitrogen, etc should be located as close as possible to the operating theatre or dissection room; if not in the actual room, then in an adjacent room and processed for freezing there at 4°C. Tissue samples stored at ambient temperatures and for in excess of 20 minutes will not be representative of the metabolome and proteome at time of sampling. Therefore processing after collection should be a rapid process. The most appropriate method is to collect tissue and place on ice during transportation and process with solutions chilled to 4°C. Cover tissue immediately with wet ice after dissection and avoid any exposure to ambient temperatures.

7.1.2. Use high quality plastics so that there is no leakage of plasticisers and contaminants into blood samples.

7.2. TISSUE COLLECTION (for metabolomics only)

7.2.1. Wash samples immediately in ice cold (4°C) saline solution (0.85% NaCl in water) in order to remove residual blood that would otherwise contaminate the tissue metabolome. As much blood should be washed from the tissue as possible within a 30 second timeframe.

7.2.2. If possible, weigh samples rapidly to determine tissue wet weight. Alternatively weigh the collection tube empty, then containing the tissue and calculate the difference.

7.2.3. Place in a suitable container and either snap freeze in liquid nitrogen or place in a -80°C freezer to freeze the tissue sample more slowly.

7.2.4. Store samples at -80°C and transport on dry ice.

7.3. TISSUE COLLECTION (for proteomics only)

7.3.1. Tissue preparation should be performed as quickly as possible in order to minimise the chances of protein degradation between harvesting and storage.

7.3.2. Once the appropriate tissue is obtained, wash the tissue well twice in ice-cold phosphate buffered saline (PBS), and then in ice-cold 0.25M sucrose to remove blood and salts. A good way to do this is to add ice-cold PBS to a Petri dish and rinse the tissue piece, holding it with a pair of forceps. Remove the tissue to a second PBS-containing dish, then finally into a dish of sucrose.

7.3.3. Touch the tissue against some tissue paper to remove any liquid and place into a storage tube (microcentrifuge tube or cryovial). Steps 7.7.2 and 7.7.3 are CRITICAL to remove as much blood as possible in order to reveal tissue proteins.

7.3.4. Weigh the tissue piece. Alternatively weigh the collection tube empty, then containing the tissue and calculate the difference.

7.3.5. Snap freeze tissue in liquid nitrogen.

7.3.6. Store samples at -80°C and transport on dry ice.