

Standard Operating Procedure

Processing and temporary storage of blood samples at BDN centres

SOP: SP001, version ^ 2

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Changes to previous version

[^] Changed text is shown in red and deletions are shown as [^]. Details of significant changes are shown under History, below.

Introduction and aim of the procedure

This standard operating procedure defines the processing at the donation centre of blood samples donated to onCore UK's tissue bank. Blood is a very accessible research material from patients and may be the only source of normal case-linked material available. Blood samples available for the onCore UK tissue bank must be collected and stored in a form that will allow for changing research requirements in the future. onCore UK aims to store whole blood, serum, plasma, buffy coat and red cells. Standardisation of the collection and storage procedures will ensure that not only the whole blood but also the bioproducts are of the highest possible quality and modifications according to demand can be easily incorporated.

Applications and restrictions

Blood shall be processed only within a containment level 2 laboratory (as defined in ACDP¹ guidelines). Blood shall be processed only by staff members who are trained in this procedure and in local health and safety procedures for working with human blood.

¹ Advisory Committee on Dangerous Pathogens: Biological agents: Managing the risks in laboratories and healthcare premises

Donors must be enrolled into the onCore UK database before beginning to process blood. Instruction on the use of the database are given in the ^ CELL ^ System Manual, SOP: IT001 and enrolment of donors is described in SOP: PC003.

Blood processing must begin as soon as possible and ideally within 30 minutes of collection from the donor.

Training

Training in use of a pipette, laminar flow cabinet, ^ centrifuge and in health and safety shall be given and recorded according to local procedures. Training in sample processing and ^ use of the ^ CELL system^ shall be given according to SOP: TR001 and recorded using form: SP001.01. A list of competent staff may be held at the^ hospital site using form: SP001.02 if that is helpful locally. ^

Associated procedures and supporting documents

^ SOP: TR001	General training procedure for onCore UK activities
Form: SP001.01	Training record - Processing and temporary storage of blood samples at BDN centres
Form: SP001.02	Record of competence - Processing and temporary storage of blood samples at BDN centres.
SOP: PC003	Enrolment of donors to the onCore UK CELL database
SOP: SP003	Use of Mr Frosty in controlled rate freezing
SOP: QS001	Deviations from standard operating procedures
SOP: ST001	Dispatch of samples from BDN centres to the onCore UK repository
IT001	CELL system manual

Definitions

Centrifugal force (g). The centrifugal force in a centrifuge is dependent on the rotating speed and the diameter of the rotation. If a given centrifugal force is needed and the rotation characteristics are known, it is possible to calculate the speed required to attain the needed force:

relative centrifugal force in gravities (g) =

$$0.00001118 \times (\text{rotating radius}) \times ((\text{rotating speed})^2)$$

where:

- The rotating radius is in centimeters. This is the horizontal distance from the center of the rotor axis to the tip of the centrifuge tube.
- The rotating speed is in revolutions per minute (rpm).

The rotating speed needed to achieve a given centrifugal force =
square root (89445 x (relative centrifugal force desired) / (rotating radius))

Health and Safety

All human biological material is considered a biohazard and handled using universal precautions according to local H+S rules. **Gloves must be worn when handling FTA Elute cards.**

Materials and equipment

^ Laminar flow cabinet ^
-80°C freezer
Centrifuge with sealed buckets or sealed rotor

500µl pipettor
 Sterile tips for pipettor
 Sterile 3ml Pastettes
 Mr Frosty, containing 250ml of isopropyl alcohol. **NB** Mr Frosty must be at room temperature at the beginning of this procedure, [see SOP: SP003](#).
 Empty cryovials with coloured lids (12 yellow, 12 white (semi-transparent), 6 red and 8 blue)
 48 place ^ plate^ to hold cryovials in the freezer^
 12 cryovials (violet lids), each containing 500µL Recovery Cell Freezing Medium² ^
 FTA Elute³ card and plastic bag
 Barcodes for FTA Elute cards
 Silica gel desiccant sachet
 ^ Two polystyrene racks to hold blood tubes and cryovials during processing
 Smartscan Solo 2D barcode reader
 Linear barcode scanner
 Computer with internet access.

Procedure

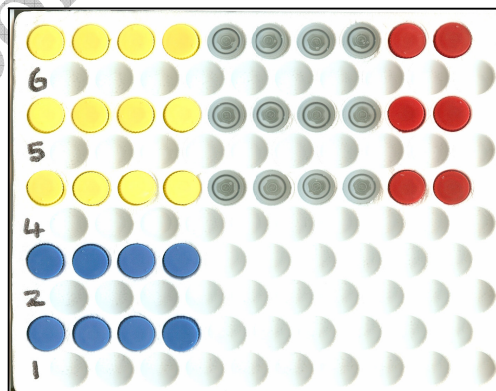
Begin processing tubes within 30 minutes of collection whenever possible. Follow local procedures for handling biological materials; treat all samples as potentially infectious.

Remove twelve cryovials (violet lids) containing cryoprotectant from the freezer and allow to thaw. ^ The liquid in these cryovials should be yellow/orange when frozen and may change to pale pink/red in colour once it is thawed; discard any cryovials that are dark pink/red.

Ensure that a Mr Frosty container is at room temperature. In an emergency place the container at 37°C for about 15 minutes to warm up. Use of the Mr Frosty is described in SOP: SP003.

Set up the ^ blue, yellow, white and red cryovials in a polystyrene rack as shown in diagram 1:

Diagram 1: Cryovial layout



² Recovery Cell Freezing Medium, Cat.No VX12648010, Thermo Fisher Scientific. Pre-aliquotted by onCore UK.

³ FTA Elute Microcards, Cat.No. WB 120410, Whatman International Ltd.

Check that donor is enrolled into onCore UK database, if not enrol donor as described in ^ SOP: PC003.

^ 1 Registration of Vacutainer tubes in database

In the “Samples” page of the CELL database, select “add blood samples”. All interactions with the onCore UK database are described in the CELL system manual (IT001) provided by onCore UK.

Use the magnifying glass symbol to look up the list of patients. Select the correct patient from the list presented, ensuring that patient’s name and date of birth match those on the blood tubes. Press “Next”.

Enter the blood collection time and processing start time.

Enter details of ALL of the equipment and reagent batches that will be used in processing the vacutainer tubes and creating derivatives. When complete, press “Next”.

Enter the volume collected in the vacutainer for each tube in turn. Take care to match the number (1-6), shown on the tube, with the number in the database as shown in table 1 below. Record whether the tube is full, partially full or empty. Enter comments against any of the tubes where this is necessary.

Tube number	Screen label	Tube type
1	^ CAT Plus 1	^ CAT Plus tube (^ red cap)
2	^ CAT Plus 2	^ CAT Plus tube (^ red cap)
3	ACD 1	ACD (yellow cap)
4	EDTA 1	EDTA tube (purple cap)
5	EDTA 2	EDTA tube (purple cap)
6	EDTA 3	EDTA tube (purple cap)

^ When complete press “Save”, wait for “Operation successful” message then press “Return to list”. This will return you to the sample list page.

Proceed according to tube type. All work with samples must be performed aseptically.

2 ^ FTA Elute cards

Wear gloves and work within a laminar flow cabinet. Note that FTA Elute cards are designed to store DNA. Care must be taken to avoid touching the sample area of the card to prevent contamination and because it is impregnated with chemicals designed to break down cells.

Obtain an FTA Elute card, attach FTA Elute barcode label to card.

Select vacutainer numbered 4 (EDTA 1), mix the sample by inverting twice and remove 0.5ml of blood using a 3ml sterile pastette. Place 2 drops of blood from the pastette onto each circle on the FTA Elute card. Do not return any blood left in the pastette to the Vacutainer tube; discard safely with the pastette.

From the sample list page of the CELL database, select tube numbered 4, labelled EDTA 1 in the database (ensure that the correct patient is selected if there is more than one set of samples to be processed).

Press “create card” ^ and scan the linear barcode into the database. This will allow the donor’s identifier to be associated with the card’s barcode. If necessary enter any comments against the FTA Elute card’s record. Press “Save” and when you see the “Operation Successful” message, press “Return to list”.

^ Place the card at the back of a ^ laminar flow cabinet or into a clean, dry area and allow it to dry for 6 to 24 hours at room temperature. Once dry, place the card into a small plastic bag, add a sachet of silica gel desiccant⁴ and store IN THE DARK at room temperature to await transport to the repository. Record storage of the FTA Elute card on the CELL database as shown in section 10.

^ 3 Centrifugation

Re-cap tube number 4.

Place all three^ EDTA tubes (numbered 4, 5 and 6) and the two CAT Plus tubes (numbered 1 and 2) into a centrifuge, ensure it is balanced, and spin at approximately 1500g for 10 (±2) minutes with the brake turned off. ^

Remove the tubes carefully from the centrifuge to avoid disturbing the cells. Place into a polystyrene rack in numerical order (1, 2, 4, 5 and 6) alongside the relevant cryovials as shown in diagram ^

^ 4 Serum

Serum is obtained from the CAT Plus tubes (red caps) numbered 1 and 2. Use a sterile pastette to remove all of the serum from tube 1 and place 1ml aliquots into up to four cryovials with blue lids in the row behind tube 1.

Discard the pastette safely according to local H+S rules.

Repeat for the second serum tube, numbered 2.

5 Plasma

Plasma, buffy coat and red cells are all obtained from the EDTA tubes. Use a sterile pastette to remove all of the plasma from tube 4 and place 1ml aliquots in^ up to four cryovials with yellow lids in the row behind tube 4. Be careful not to disturb the buffy coat.

^ Discard the pastette safely according to local H+S rules.

^ 6 Buffy coat

Use a new sterile pastette to remove the buffy coat ^ slowly, using a circular motion to pick up all visible buffy coat which is greyish-white in colour. Note that the buffy coat adheres to the red cell layer; some red cells and plasma will be drawn up with the buffy coat.

^ Divide the buffy coat equally between four cryovials with white lids in the row behind tube 4. There should be a minimum of two drops in each cryovial.

Discard the pastette safely according to local H+S rules.

⁴ Desiccant should be blue in colour. Sachets that have turned pink can be regenerated by heating in an oven until blue and cooling to room temperature before use.

^

7 Red cells

Use a new pastette to remove the remaining red cells and place 1ml aliquots into two cryovials with red lids in the row behind tube 4.

Discard the pastette safely according to local H+S rules.

Repeat steps 5, 6 and 7 for the EDTA tubes labelled 5 and 6^ .

8 Recording cryovials in CELL database.

Turn on the Smartscan Solo as shown in the CELL System Manual, IT001. Ensure that the associated dongle is inserted into the correct USB port on your computer; the same port must be used every time this scanner is used.

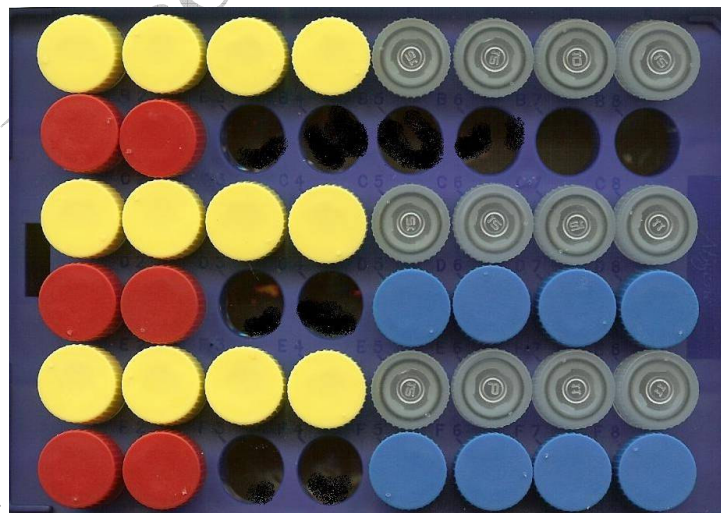
From the sample list page of the CELL database, select CAT Plus 1 (ensure that the correct patient is selected if there is more than one set of samples to be processed), select "Create derivative samples", scan the barcode of each of the serum cryovials filled from tube 1 into the appropriate field. Record the fill volume for each cryovial and add any extra information or comments to the "Notes" fields.^ NOTE THAT these fields will be printed onto the Shipping Manifest and must not contain any patient identifiable data. Press "Next".

^ Amend the processing end time field if necessary (default is the current time). . Press "Save" wait for the "operation successful" message and then "Return to list".

Repeat for the other CAT Plus and EDTA tubes, ensuring that the correct tube is selected from the sample list page and the correct cryovials are scanned against that tube.

Place the cryovials into a 48 place plate in the layout shown in diagram 2 below. DO NOT PLACE CRYOVIALS FROM ANY OTHER DONOR INTO THAT PLATE. At the repository, cryovials from each donor will be split for storage at either -80 or in vapour phase liquid nitrogen. Sample sorting is far easier if all of the samples from a single donor are in the same plate.

Diagram 2: Plate layout for storage of samples from one donor at -80



Discard the Vacutainer tubes according to local procedures for waste disposal.

9 Whole blood

Whole blood is obtained from the acid citrate dextrose (ACD) tube (yellow cap). This tube can be processed while the EDTA and CAT Plus tubes are spinning, or left until the other tubes have been processed. It is essential that the ACD tube is processed completely without breaking off once processing starts since the cryoprotectant is toxic to cells at room temperature.

Ensure the twelve cryoprotectant tubes (cryovials with violet lids) are thawed.

Mix the ACD tube (tube 3) by inverting twice.

Use a calibrated pipette with a sterile tip to add 500µl of whole blood to each of the cryoprotectant tubes and mix by inverting five times. Discard the tip from the pipette and the ACD tube according to local procedures for waste disposal.

From the sample list page of the CELL database, select the ACD 1 tube (ensure that the correct patient is selected if there is more than one set of samples to be processed), select "Create derivative samples", scan the barcode of each of the whole blood cryovials filled from tube 3 into the appropriate field. Record the fill volume for each cryovial and add any extra information or comments to the "Notes" fields. NOTE THAT these fields will be printed onto the Shipping Manifest and must not contain any patient identifiable data. Press "Next".

Amend the processing end time field if necessary (default is the current time). Enter any notes against individual cryovials if necessary. Press "Save" wait for the "Operation successful" message and then press "Return to list".

^

Place the cryovials into the Mr Frosty container (see SOP: SP003, Use of Mr Frosty in controlled rate freezing) and **immediately** transfer to -80°C freezer overnight (at least eight hours), then transfer samples from Mr Frosty to a plate inside the -80°C freezer to await transport to the repository. Use a separate plate to that used for the other cryovials; violet cryovials from several donors may be placed in the same plate.

Place empty Mr Frosty at room temperature to warm up so that it can be re-used.

10 Storage

Once all of the cryovials have been placed in storage plates as appropriate, transfer them to the -80 freezer to await transport to the repository according to SOP: ST001. Note that all cryovials are stored at -80 at this stage; a portion of the cryovials will be transferred to liquid nitrogen storage at the repository.

^

Select "Sample storage" in the CELL database, select the "Non-frozen to store" query, select the FTA Elute card, press "Store samples". Select the local room temperature storage unit using the "lookup" magnifying glass, select the FTA Elute card, press "Autofile", press "Save" and, once the operation is successful, press "Return to list". Select the "Frozen to store" query and repeat for the cryovials, selecting the local -80 freezer. Once complete, log off as shown in the CELL system manual (IT001).

The procedure for creating a package and sending to the central repository is given in SOP: ST001 Dispatch of samples from BDN centres to the onCore UK repository.

^ 11 Deviations

Any deviations from this procedure shall be recorded and investigated according to SOP: QS001.

History

Version	Date issued	Changes to previous version
1	28 Jun 2007	None, new procedure
2	06 May 2008	Amendment to distribution list. Full re-write of procedure; re-training of operators needed.

END

Uncontrolled when printed