	SOP Number:	DONOR SOP001
	Version Number:	7.1
	Work Package	4
	Co-ordinating Countries:	Groningen/ Maastricht
	Approval Date:	16/02/2015
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	Approver:	Maria Kaisar

Title

Collection, processing and storage of donor biological samples for COPE-COMPARE Trial.

You are instructed to read the following thoroughly before proceeding to undertake the methods described.

Under no circumstances are these instructions to be amended or altered in any way other than by the author / approver.

Summary of Significant Changes

Date	Details of Review	Version Number	No. of pages	Reviewer	Next Review
16/02/2015	Minor corrections to DONOR SOP001	DONOR SOP001 7.0	11	Miss Bhumika Patel	6 months

Purpose

The purpose of this document is to inform and guide the donor transplant team in obtaining biological samples for COMPARE trial and donor clinical data. The COMPARE trial aims to address whether oxygenated is superior to non-oxygenated machine perfusion in increasing the longevity and quality of older DCD kidneys. This document describes the procurement of blood, urine and perfusate samples obtained during the donation procedure. This trial will explore the potential regeneration properties of O_2 in HMP.

Abbreviations

COPE	Consortium of Organ Preservation in Europe
DB1.1	Donor blood 1.1
DB1.2	Donor blood 1.2
DU1	Donor urine 1
DU2	Donor urine 2
LKP1	Donor left kidney perfusate 1
LKP2	Donor left kidney perfusate 2
RKP1	Donor right kidney perfusate 1
RKP2	Donor right kidney perfusate 2
TT	Transplant Technician

Summary of sample collection

Responsibilities

Researcher – to make sure specific consent for the COMPARE-COPE trial has been gained before obtaining samples.

Scrub Nurse – to assist in the sample collection where needed.

Anaesthetist. -To obtain the blood & urine samples

Transplant technician (TT) –

1. To collect the Donor COPE WP4 Box from COPE co-ordinating centre (Groningen / Maastricht) and the laptop and hand scanner.
2. To check prior to departure from COPE co-ordinating centre that the COPE WP4 Donor Box contains the proper barcoded sample tubes.
3. On arrival to the donor hospital the TT will assist in sample collection of DB1, DU1, DU2, LKP1, LKP2, RKP1, RKP2 , centrifuge the blood, urine and perfusate samples and transfer to new tubes according to the SOPs.
4. To ensure that the perfusate samples are stored and transferred with the appropriate kidney to the recipient hospital.
5. To scan the sample barcodes and fill the relevant fields in the database.
6. To assemble the COPE WP4 box with all the samples and place it on ice during transfer to COPE co-ordinating centre (Groningen / Maastricht)
7. To store the samples in -80°C upon arrival in the COPE co-ordinating centre (Groningen / Maastricht)
8. To organise the sample transportation to Oxford every three months according to the protocol.

Samples for collection

Donor

Blood & urine samples

DU1- 1st urine sample obtained at withdrawal of support.

DB1- 1st set of blood samples obtained from the central line at withdrawal of support.

DU2- 2nd urine sample obtained from the last urine collected from the small chamber catheter bag

During HMP-K

Perfusate samples HMP-K (with/without O2)

Perfusate Samples are obtained from both kidneys.

RK P1 – Right Kidney:1st Perfusate sample taken 15 min after the commencement of HMP-K.

LK P1 –Left Kidney: 1st Perfusate sample taken 15 min after the commencement of HMP-K.

RK P2 –Right Kidney: 2nd perfusate sample taken before leaving the hospital.

LK P2 – Left Kidney: 2nd perfusate sample taken before leaving the hospital. This sample is transferred to the recipient hospital and stored in the recipient COPE box..

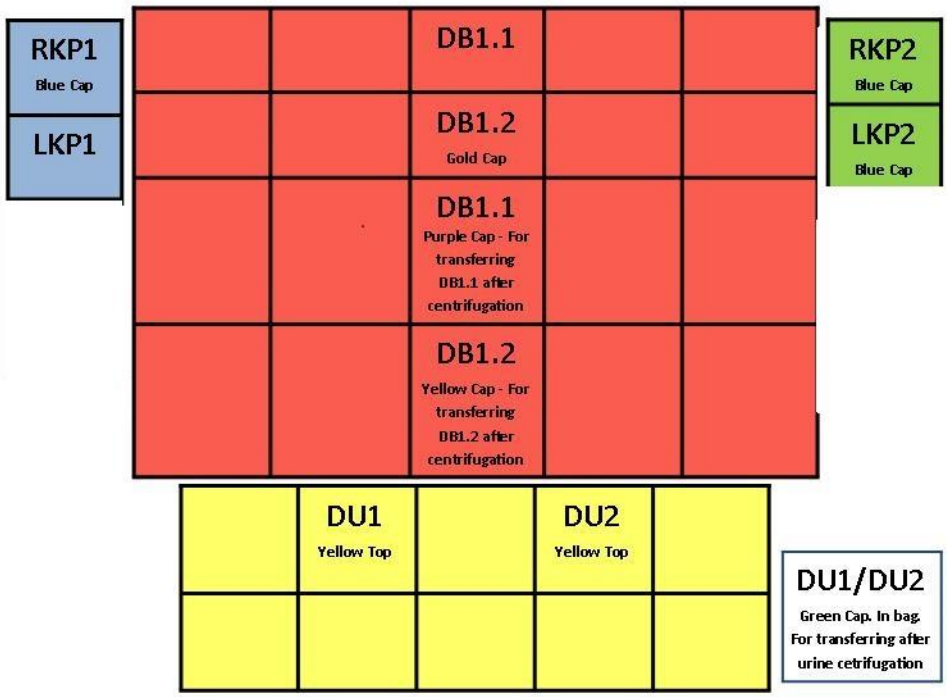
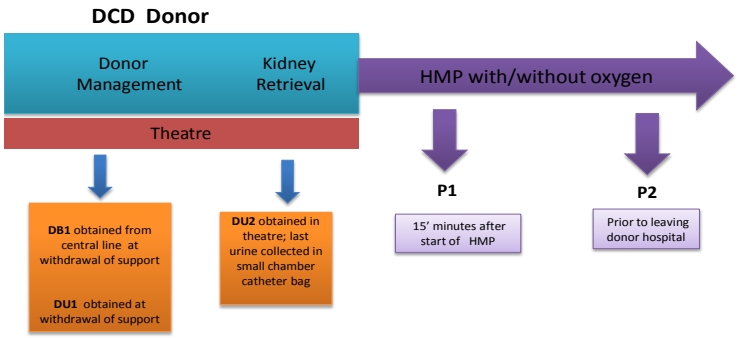


Fig. 1 Sample map as appears on the COPE WP4 Donor box

WP4: **$HMPO_2$ vs HMP in Kidney Transplantation**



P: perfusate; **DB**: donor blood; **DU**: donor urine; **RB**: Recipient blood; **L/R KT** : Left/right kidney tissue biopsy

Fig. 2 Biological sample collection points in HMPO2 *vs* HMP in Kidney transplantation

Safety Information
1. Comply with the local health and Safety rules at all times. 2. All samples must be treated as potentially infectious. 3. Personal Protective Equipment (gown / labcoat, gloves) must be worn when handling samples.

Attention is needed
To identify the correct sample containers from the COPE WP4 Box prior the sample collection To accurately fill in the appropriate section of the COPE worksheet.

1. Blood & urine sample collection

1.1. DU1 urine sample

A 4 ml urine sample is obtained at withdrawal of support

1. Identify the DU1 sample container in the COPE WP4 Donor Box.
2. Obtain 1x 4ml urine sample in the container labelled DU1.
    a. Empty the urine container into the urine bag after taking DU1
3. Centrifuge the urine sample according to the instructions at section 4. Use a counter weight tube to balance the centrifuge.
4. Transfer the urine after centrifugation to a new tube labelled DU1 using the plastic pipette provided in the COPE Box.
5. Scan the barcode to the database
6. Fill all the appropriate fields in the database.
7. Place the DU1 sample in the ice container until transport.

2. DB1 blood sample

A 6 ml EDTA (DB1.1, white cap) and a 6 ml SST (Serum) (DB1.2, yellow cap) sample are obtained from the central line at withdrawal of support by the anaesthesiologist.

1. Identify the DB1.1 & DB1.2 sample containers in the COPE Box.
2. The anaesthetist obtains the blood sample from central line prior to withdrawal of support.
3. The anaesthetist's hands out the syringe with the blood to the transplant technician who fills the appropriate 1x6ml DB1.1 & 1x 6ml DB1.2 blood containers to the minimum level.
4. Tubes should be inverted gently at least 5 times to insure proper mixing with the anticoagulant.
5. Centrifuge the blood samples according to the instructions at section 4.
6. Transfer the cell free plasma to DB1.1 (purple cap) and the serum cell free sample to DB1.2 (yellow cap) using the plastic pipettes in the COPE box.
7. Scan the barcode to the database
8. Fill all the appropriate fields in the database.
9. Place the tubes in the ice container until transport.

3. DU2 urine sample collection

A 4 ml urine sample is obtained in the theatre from the last urine collected in small catheter bag.

1. Identify the DU2 sample container in the COPE WP4 Donor Box.
2. Obtain 1x 4ml urine sample in the container labelled DU2
3. Centrifuge the urine sample as described in section 4 and transfer the urine to the new tube DU2.
4. Scan the barcode of DU2 tube in the database.
5. Fill the appropriate fields in the database
6. Keep the centrifuged sample in ice with the rest of the samples

4. Centrifugation of blood & urine samples

1. Take the centrifuge out of the bag, place on a level surface and plug in.

Blood and urine tubes that have been kept at room temperature (RT) are placed in the centrifuge. Extra tubes for balancing will be found in the COPE box. These tubes can be filled with water to balance the centrifuged.

2. The serum, SST (yellow top) should have been allowed to clot. This will take 30 min.
3. Centrifuge at 1300G/RCF for 15 minutes at room temperature.
4. Once centrifuged, remove the tubes from the centrifuge and place on the bench.
5. Transfer the plasma from the DB1.1 to the new empty tube labelled DB1.1 using the plastic pipette provided.
6. Transfer the serum DB1.2 in the appropriate labeled empty tube DB 1.2 using the plastic pipette provided.
7. Place the new tubes in the correct location according to the map in the COPE WP4 Donor box.
8. Transfer the DU1 urine sample with the plastic pipette provided in the new empty tubes DU1 located in the small bag.

9. Store the COPE WP4 Donor box with samples on ice.
10. Make sure all the appropriate fields in the database filled before added in the box.

5. Collection of perfusate samples

5.1 Perfusate P1 sample (RKP1, LKP1)

A perfusate sample 1x6ml from each kidney (LKP1, RKP1) obtained 15 min after the commencement of HMP.

Perfusate samples are collected from both kidneys

1. Identify the RKP1 perfusate sample containers.
2. Take perfusate sample RKP1 from the Kidney assist using following steps (Fig. 3):
    a. On top of the oxygenator is a two-way valve which is closed by a cap. Remove this cap.
    b. Put a syringe on the valve.
    c. Open the valve and take the perfusate sample.
    d. Close the valve.
    e. Remove the syringe and close the valve with the cap.
3. Fill the tube from the perfusate of the right kidney. Identify the LKP1 perfusate sample containers, repeat step n° 2 and fill the tube from the perfusate of the left kidney. Ensure the containers are filled to the minimum level.
4. Document the time of the sampling in the database.
5. Place these samples on ice in the cold box used to keep the perfusion fluid cold.

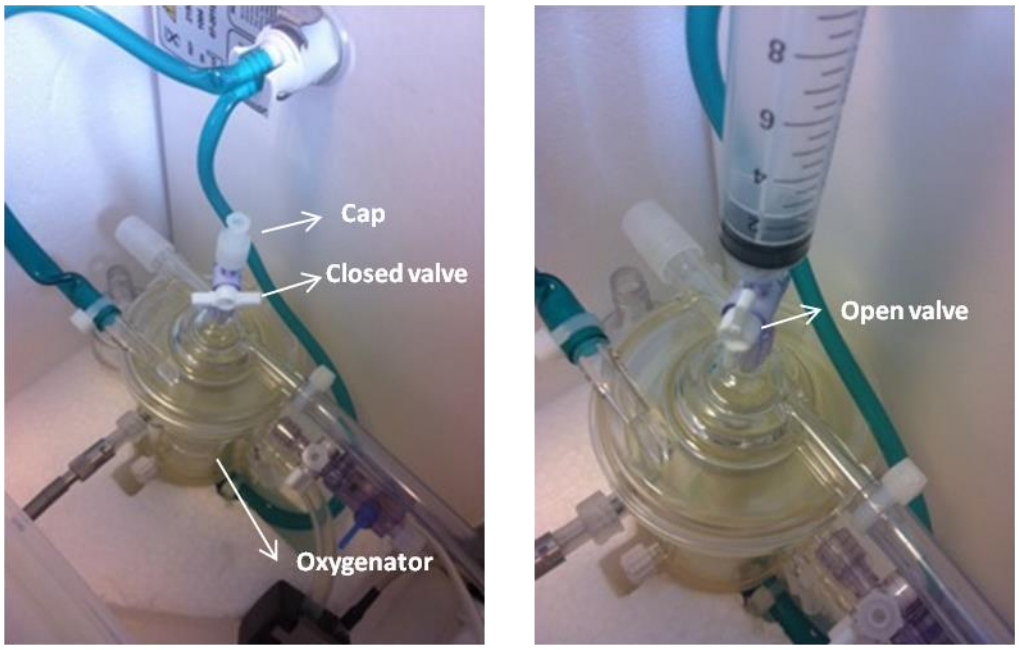


Fig. 3 Obtaining a perfusate sample from the Kidney Assist

5.2 Perfusate P2 sample (RKP2, LKP2)

A perfusate sample 1x6ml from each kidney (LKP2, RKP2) obtained before leaving the hospital.

Perfusate samples are collected from both kidneys

1. Identify the RKP2 perfusate sample containers.
2. Take perfusate sample RKP2 from the Kidney assist using following steps (Fig. 3):
    a. On top of the oxygenator is a two-way valve which is closed by a cap. Remove this cap.
    b. Put a syringe on the valve.
    c. Open the valve and take the perfusate sample.
    d. Close the valve.
    e. Remove the syringe and close the valve with the cap.
3. Fill the tube from the perfusate of the right kidney Identify the LKP2 perfusate sample containers, repeat step n° 2 and fill the tube from the perfusate of the left kidney. Ensure the containers are filled to the minimum level.

4. Scan the perfusate sample barcodes to the database

4. Document the time of the sampling in the database.

5. Store RKP2 and LKP2 on ice in the cold box used to keep the perfusion fluid cold.

6. Transportation of perfusate samples P1 & P2 to the recipient hospital with the corresponding kidney

Scan the perfusate sample barcodes to the database

1. The perfusate samples LP1 & LP2 will follow the left kidney.
2. The perfusate samples RP1 & RP2 will follow the right kidney.

7. Sample checking prior to departure from donor hospital:

1. Check that all tubes (blood, urine and perfusate) have been tightly closed to avoid spillage during transport.
2. COPE WP4 Donor Box should contained(Fig 4)
    a. DU1
    b. DB1.1 & DB1.2
    c. DU2
3. Ensure replenishment of ice in ice-box prior to departure.
4. Ensure replenishment of ice in ice-container of the perfusion fluid of the perfusion machine prior to departure
5. Ensure that all perfusate samples have been placed with the corresponding kidneys in the OrganAssist perfusion machines.
6. If any spillage/leakage occurs during transportation use a pair of gloves and close the leaking container. Notify the Regional Co-ordinator on arrival or by leaving information on the COPE sample login sheet.

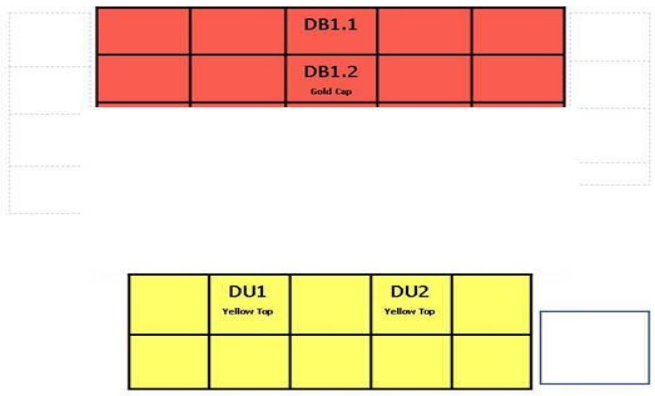


Fig. 4 Sample map demonstrates the samples that should be contained in the COPE WP4 donor box on departure from donor hospital

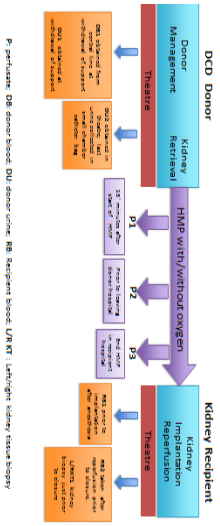
8. On arrival to COPE regional centre

1. Obtain the COPE Box.
2. Identify each sample against the information stored in the database.
3. If the database with donor information has not been completed, complete the database with donor information and scan all the samples according to the database settings.
4. Place the blood (DB1) and urine (DU1 and DU2) in the appropriate racks and store in -80°C until transfer to Oxford bio-resource.

M1

Deviations from the Standard operating protocol

Deviation	Describe deviation	Date/ Time	Signature/ Name



Version 1.0

COPE WP4 HMP-K Donor Worksheet

WP4: $HMPO_2$ vs HMP in Kidney Transplantation

	Input COPE Barcode here
COPE Centre:	
Date of Donation	
Unique Recipient Identifier for Left Kidney	
Unique Recipient Identifier for right Kidney	
Euro-Transplant Number or NHSBT- number	
Kidney Indicate the procurement of one or both kidneys	Right / Left/ both
Transplant technician name and e-mail address	

Samples description	Samples	Date collection	Time collection	Collected (V)	Time of centrifugation	Comments
Urine obtained at withdrawal of support	DU1.1					
Blood obtained from central line at withdrawal of support	DB1.1 EDTA DB1.2 SST					
Urine obtained at theater, last urine collected in catheter bag	DU2.1					
1st Right kidney Perfusate	RKP1					
1st Left Kidney perfusate	LKP1					
2nd Right kidney perfusate	RKP2					
2nd Left kidney perfusate	LKP2					

Fig. 4 **Worksheet overview**