| | | |
|---|---|---|
| | **SOP Number:** | RECIPIENT SOP001 |
| | **Version Number:** | 6.2 |
| | **Work Package** | 4 |
| | **Co-ordinating Countries:** | Oxford/Groningen / Maastricht / Leuven |
| | **Approval Date:** | 06/08/2015 |
| | **Author:** | Maria Kaisar |
| | **Approver:** | Maria Kaisar |

***Title:*** **Collection, processing and storage of recipient biological samples for COMPARE – COPE 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 RECIPIENT SOP001 | RECIPIENT SOP001 6.0 | 10 | Miss Bhumika Patel | 6 months |
| 26/05/2015 | Clarification of RNAlater sample handling instructions | RECIPIENT SOP001 v.6.1 | 10 | Bhumika Patel | 6 months |

***Purpose***

The purpose of this document is to inform and guide the donor transplant team in obtaining biological samples and clinical data for the COPE-COMPARE Trial. The 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 recipient procedure. This trial will explore the potential regeneration properties of $O_2$ in HMP.

**Abbreviations**

COPE Consortium of Organ Preservation in Europe
RB1.1 Recipient blood 1.1
RB1.2 Recipient blood 1.2
P1 Perfusate 1
P2 Perfusate 2
P3 Perfusate 3
P3.1 Perfusate transfer tube
RK1Fo Reperfusion kidney formalin
RK1Rn Reperfusion kidney RNAlater
TT Transplant Technician

| **Responsibilities** |
| --- |
| **Researcher** – to make sure specific consent for the COPMARE-COPE trial has been gained before obtaining samples.<br>**Scrub Nurse** – to assist in the sample collection where needed.<br>**Surgeon -**.To obtain the blood & biopsy samples<br>**Transplant technician (TT)** –<br>**1**.To collect the recipient COPE WP4 Box from COPE co-ordinating Groningen or Maastricht<br>**2**. To check prior to departure from COPE co-ordinating centre that the COPE WP4 Recipient Box contains the proper barcoded sample tubes.<br>**3**.To collect the hand scanner from the COPE co-ordinating centre Groningen or Maastricht<br>**4**. On arrival to the recipient transplant hospital the TT will assist in sample collection of RB1, RB2 ,P3 and tissue biopsies L or RKT1, centrifuge the blood and perfusate samples and transfer to new tubes according to the SOPs, scan the barcodes of samples in the COPE WP4 database.<br>**5.** To complete all the required fields in the database.<br>**6.** To assemble the COPE WP4 box with all the samples and place on ice during transfer to COPE co-ordinating centre in Groningen or Maastricht<br>**7.** On arrival to COPE co-ordinating centre in Groningen or Maastricht to store the samples in -80C |

**Samples for collection**

**Recipient**

**Perfusate**

**P3- 3rd** Perfusate sample taken towards the end of HMP-K on arrival to the recipient transplant hospital.

**Blood samples**

**RB1**- 1st set of blood samples obtained after anaesthesia prior to kidney implantation.

**RB2**- 2nd set of blood samples obtained after reperfusion prior to closure.

**Tissue reperfusion biopsies**

**RKT** – Reperfusion

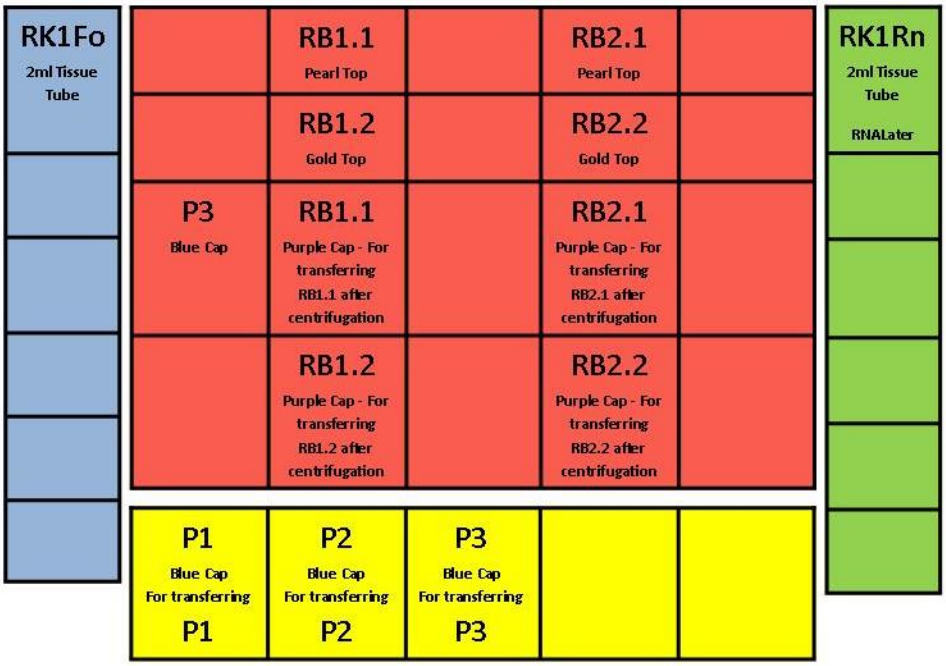



Fig 1. Sample map as appears on the COPE WP4 right kidney recipient box

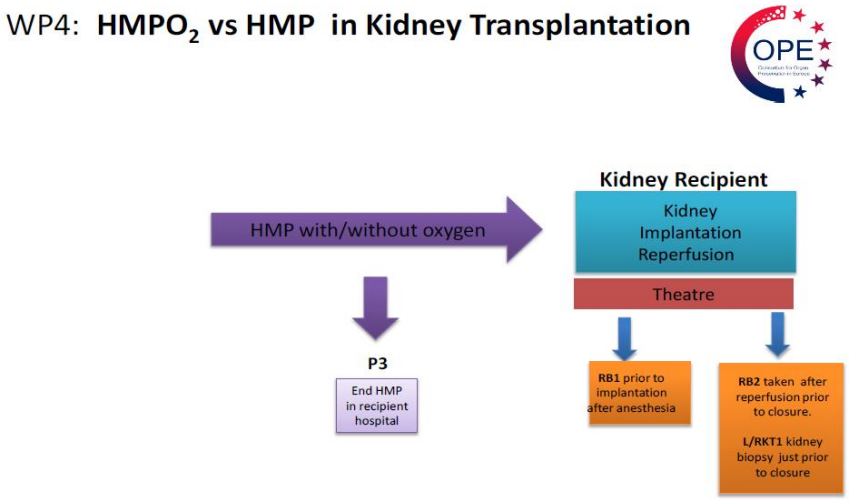



**Fig 2. Biological sample collection points in HMPO2 vs HMP in Kidney transplantation**

| ***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*** |
| --- |
| 1. To identify the correct sample containers from the COPE WP4 Box prior the sample collection<br>2. To identify and enter in the database if the kidney is left or right.<br>3. To fill accurately the appropriate section of the COPE<br>4. |

***Identify the P1 & P2 perfusate samples that arrived with the OrganAssist perfusion machine.***

***These samples should be located in the ice container that keeps the perfusion fluid cold. P1 &P2 will be processed at the same time as P3.***

## 1. Collection of perfusate samples

### 1.1 Perfusate sample P3

A perfusate sample 1x6ml from the left or right kidney (P3) obtained at the end of HMP on the arrival of the kidney in the recipient hospital.

**Confirm and enter in the database if the kidney is left or right**

1. Identify the P3 perfusate sample containers and fill the tube as follow. Fig 2
    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.

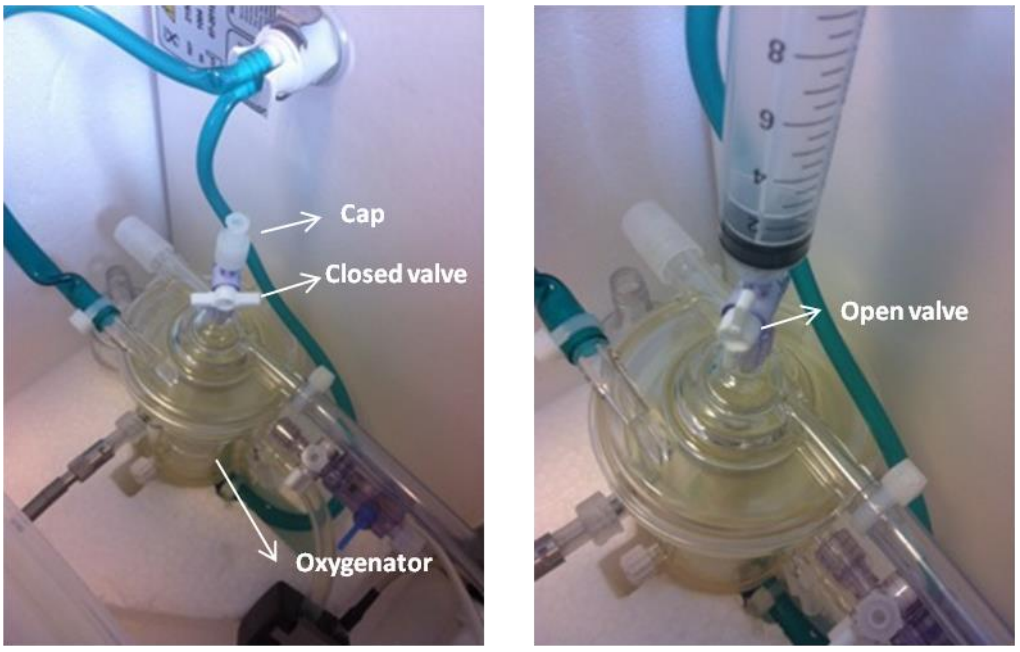



Fig. 2 Obtaining a perfusate sample from the Kidney Assist

2. Fill the tube from the syringe.
3. Ensure the containers are filled to the minimum level.
4. Document the time of the sampling in the database.
5. Place these samples back into the P3 section of the COPE box and store on ice in the cold box used to keep the perfusion fluid cold.

## 2. Blood samples procurement

### 2.1 RB1 blood sample

1. A 6 ml EDTA (RB1.1, whitish cap) and a 6 ml SST (Serum) (RB1.2, yellow cap) samples are obtained after anaesthesia and prior to kidney implantation.

2. Identify the RB1.1 & RB1.2 sample containers in the COPE Box

3. The anaesthetist hands out the syringe with the blood to the transplant technician who fills the appropriate 1x6ml RB1.1 & 1x 6ml RB1.2 blood containers to the minimum level.

4. Tubes should be inverted gently at least 5 times to insure proper mixing with anticoagulant.

5. Allow 30 min for the serum sample to clot.

6. Centrifuge the blood and perfusate samples.

7. Transfer the samples to the new tubes provided in the box with plastic pipettes.

8. Fill the appropriate section in the database

9. Keep the COPE WP4 Recipient box in ice.

## 3. Centrifugation of blood samples & perfusate

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

2. Blood tubes that have been kept at room temperature (RT) are placed in the centrifuge. The number of tubes that have been collected until now are blood: RB1.1, RB1.2 and perfusate samples:

    - Perfusate arrived from donor hospital P1&P2 and
    - Perfusate samples obtained at the recipient hospital P3

3. The blood and perfusate samples will not balance the centrifuge so will have to use the counterbalance tubes found in the COPE Recipient Box.

4. The serum, SST (yellow top) should have been allowed to clot. That will take 30 min.

5. Centrifuge at 1300G/RCF or 3700 RPM for 15 minutes at room temperature.

6. Once centrifuged, remove the tubes from the centrifuge and place on the bench.
7. Transfer the plasma from the RB1.1 to the new empty tube labelled RB1.1 using the plastic pipette.
8. Transfer the serum RB1.2 in the appropriate labeled empty tube RB 1.2 using the plastic pipette.
9. Transfer P1, P2 and P3 to new tubes labelled P1.1, P2.1 and P3.1 and place on ice
10. Scan the barcodes of the perfusate and blood tubes in the database
11. Make sure the proper fields in the database are completed.
12. Assemble the COPE box with all the samples and store on ice

## 4. Blood samples procurement

### 4.1 RB2 blood sample

1. A 6 ml EDTA (RB2.1, lavender cap) and a 6 ml SST (Serum) (RB2.2, yellow cap) samples are obtained after kidney implantation and reperfusion prior to closure.
2. Identify the RB2.1 & RB2.2 sample containers in the COPE Box
3. The surgeon hands out the syringe with the blood to the transplant technician who fills the appropriate 1x6ml RB2.1 & 1x 6ml RB2.2 blood containers to the minimum level.
4. Tubes should be inverted gently at least 8 times to insure proper mixing with anticoagulant.
5. Fill the appropriate section in the database.
6. Keep the blood samples at room temperature until centrifugation.
7. Centrifuge the blood samples.

## 5. Centrifugation of blood RB2 samples

1. Blood tubes that have been kept at room temperature (RT) are placed in the centrifuge.
2. The number of tubes that have been collected until now RB2.1, RB2.2 will balance the centrifuge.
3. The serum, SST (yellow top) should have been allowed to clot. That will take 30 min.
4. Centrifuge at 1300G/RCF or 3700 RPM for 15 minutes at room temperature.
5. Once centrifuged, remove the tubes from the centrifuge and place on the bench.

6. Transfer the plasma from the RB2.1 to the new empty tube labelled RB2.1 using the plastic pipette
7. Transfer the serum RB2.2 in the appropriate labeled empty tube RB 2.2 using the plastic pipette.
8. Scan the barcodes of the blood tubes in the database
9. Place the new tubes in the correct location according to the map in the COPE WP4 Recipient box
10. Store the box with samples on ice
11. Make sure the appropriate sections in the database are completed
12. Assemble the COPE box with all the samples and store in ice

## 6. Reperfusion kidney biopsy samples

1. A Kidney biopsy is obtained by transplant surgeon after reperfusion prior to closure. Each biopsy should be handled carefully and divided to two segments one to be stored in RNAlater and the other in formalin at room temperature.
2. Document in the database if the kidney received is left or right kidney
3. Remove the outer package of biopsy gun. Surgeon assembles device, removing protective sheath surrounding biopsy needle. The size needs to be set at 23mm.
4. Surgeon inserts needle into the kidney.
5. Surgeon fires gun when ready to take the biopsy. Once fired remove needle from kidney.
6. Surgeon expels biopsy specimen onto clean wet swab.
7. Hand swab to transplant technician.
8. TT divides the biopsy specimens into two on a wet swab using a blade. One half is stored in RNAlater and the other in formalin.
9. Identify the kidney biopsy tubes filled with formalin and RNAlater in the COPE Recipient Box and place half of the biopsy in each.

10. Scan the barcodes of the tubes in the database.

11. Return tubes to the COPE box.

12. Complete appropriate sections in the database.

## 7. Sample checking. Prior to departure from donor hospital:

1. Check that all tubes (blood, perfusate and tissue samples) have been tightly closed to avoid spillage during transport.

2. COPE WP4 Recipient Box should be transferred on ice until arrival to COPE co-ordinating centre in Oxford.

3. Ensure replenishment of ice in ice-box prior to departure.

4. 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.

## 8. On Arrival to COPE regional centre

1. Obtain the COPE Box.

2. Identify each sample against the sample information on the database.

3. If the database with recipient information has not been completed, complete the database with Recipient information and scan all the samples according to the database settings.

4. Place the blood and perfusate samples in the appropriate racks and store in -80C.

5. Place the formalin biopsy in room temperature storage. Follow the instructions in the correct version of *SOP-WP7-001 Transfer of Formalin Biopsies to Ethanol,* after 24 to 27 hours, to ensure the formalin biopsy is processed and placed in ethanol for long term storage.

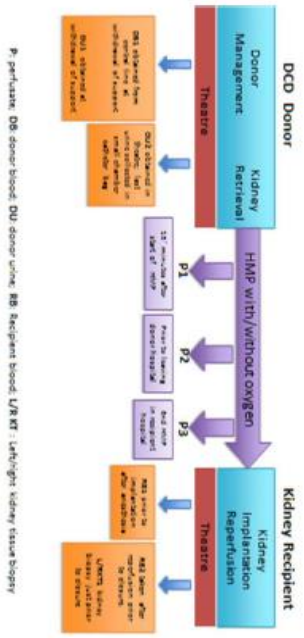
6. After 24 hours, or at the samples time of processing the formalin samples, pipette the RNAlater fluid out of the tube, and dispose in the waste container. Place in the -80C Freezer with the blood and perfusate samples, in the appropriate racks.

7. The designated person will follow instructions in the correct version of *SOP-WP7-003 Scan Barcodes of Frozen Samples into WP7 Database* to log and record the samples.

M1

**Deviations from the Standard operating protocol**

| | | | | | | Deviation | Describe deviation | Date/ Time | Signature/ Name |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |




Version 1.0

**COPE WP4 HMP-K Recipient Worksheet**

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

Input COPE Barcode here

| | |
|---|---|
| COPE Centre: | |
| Date of Transplant operation | |
| Euro-Transplant Number or NHSBT- number | |
| Kidney (please circle) | Left Right |
| Transplant technician name and e-mail address | |

| **Perfusate & Blood Samples** | Sample | Collected (Tick) | Sample collection | | Centrifugation | |
|---|---|---|---|---|---|---|
| | | | Date | Time | Date | Time |
| **P3.1 (End of HMP)** | Perfusate | | | | | |
| **P1.1** perfusate transferred from donor hospital | N/A | N/A | N/A | N/A | | |
| **P2.1** perfusate transferred from donor hospital | N/A | N/A | N/A | N/A | | |
| RB1 (Recipient blood after anaesthesia prior to implantation) | RB1.1 EDTA | | | | | |
| | RB1.2 SST | | | | | |
| Time of kidney reperfusion | | | | | | |
| RB2 (Recipient blood after reperfusion prior to closure) | RB2.1 EDTA | | | | | |
| | RB2.2 SST | | | | | |
| Reperfusion biopsy KT | RNAlater | ReKT RNAlater | | | | |
| | Formalin | ReKT formalin | | | | |

**Fig. 3: Recipient sample collection work sheet**