Sedation, Temperature and Pressure after Cardiac Arrest
and Resuscitation – the STEPCARE trial

Blood Collection and Processing Instructions for the Biomarker Substudy

Version 1.1
November 21st, 2023

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1. THE BIOMARKER SUBSTUDY

Participation in the STEPCARE biomarker substudy is optional. Sites interested in participation should complete a checklist for assessment of eligibility prior to start-up. The checklist and other documents for the biomarker substudy are published in the biomarker section of the STEPCARE website https://stepcare.org/biomarkers.

After collection, processing, and storage at the site according to Table 1, the samples will be shipped to the Integrated Biobank of Luxembourg (IBBL) for storage and analysis.

Table 1. Sample collection and processing for one patient

Time point [a]	Blood volume	Collection tubes	Processing	Aliquot labels	Sample Collection Forms
12 h	6 ml 6 ml	**1 x SST** **1 x EDTA[b]**	4 x 500 µl serum 4 x 500 µl plasma	SER -01 to 04 PLA -01 to 04	1
24 h	6 ml 6 ml	**1 x SST** **1 x EDTA**	4 x 500 µl serum 4 x 500 µl plasma	SER -05 to 08 PLA -05 to 08	1
48 h	6 ml 6 ml 2.5 ml	**1 x SST** **1 x EDTA** **1 x PAXgene RNA**	4 x 500 µl serum 4 x 500 µl plasma None	SER -09 to 12 PLA -09 to 12 N/A	1
72 h	6 ml 6 ml	**1 x SST** **1 x EDTA**	4 x 500 µl serum 4 x 500 µl plasma	SER -13 to 16 PLA -13 to 16	1
In total	**51 ml**	**4 x SST** **4 x EDTA** **1x PAXgene RNA**	**16 x 500 µl serum** **16 x 500 µl plasma**	**16 serum labels** **16 plasma labels**	**4**

[a] Hours after randomisation, +/- 8 hours are permitted if necessary

[b] The EDTA tube is saved for mitochondrial DNA isolation

2. INSTRUCTIONS FOR THE STUDY COORDINATOR

Blood samples are collected at 12, 24, 48, and 72 h after randomisation from an existing central venous or arterial access device. If laboratory processing according to the instructions is not possible outside "office hours", samples may be drawn +/- 8 hours from the designated time point. Efforts should be made to obtain blood samples as close as possible to the ideal collection time points.

2.1. Materials

Sample collection kits and storage boxes for frozen cryovials will be sent from IBBL to the study coordinator, who will make necessary preparations and distribute as appropriate. A sample collection kit contains materials for one patient, as shown in Figure 1 A. Each kit has a unique 8-digit kit ID (12345678-SC-VAR) and can only be used for one patient. A strip of kit ID labels for forms and four smaller plastic bags with material for each timepoint (12, 24, 48, 72 h) are included inside the main bag, as shown in Figure 1 B.

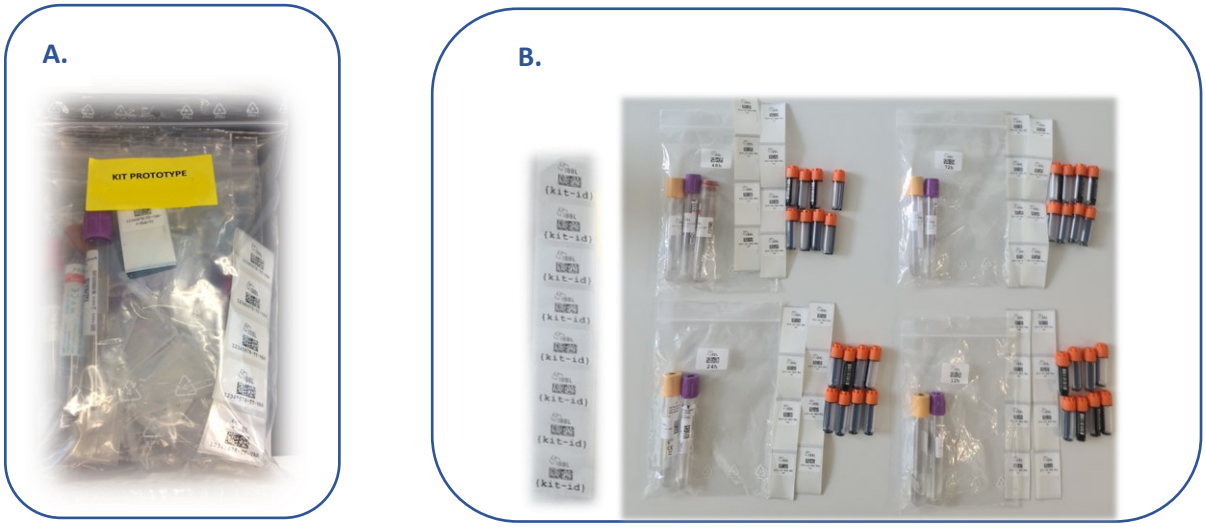

Figure 1 A. A sample collection kit for one patient. **B.** The content of a sample collection kit, including a strip of kit ID labels and 4 smaller bags for the timepoints 12, 24, 48, 72 h with blood collection tubes and materials for the laboratory (cryovials and labels for cryovials).

2.2 Preparations of sample collection kits

In addition to the materials provided by IBBL, sample collection forms must be printed by the site (Appendix A). The form can be downloaded at https://stepcare.org/biomarkers and may be translated and adapted to local routines if desired. **Four sample collection forms per patient should be printed double sided and in color.** Page 1 of the form should be completed by the ICU staff and page 2 by the laboratory staff. To facilitate for the clinical staff, we advise study coordinators to pre-label four forms/patient using the kit ID labels and insert the forms either into the main bag (Figure 1A) or the bags for each timepoint (Figure 1B).

2.3. Instructions for blood collection

Instructions for obtaining blood samples are included in the sample collection form. After preparation of the kits, the clinical staff will have the required materials and instructions in the kit bags for each patient. Please ensure that the important instructions below are followed:

- An eight-digit unique kit ID identifies each patient and should be **recorded in the patient´s medical record**. The strip with kit ID labels in the main bag could be used for this purpose.
- It is essential to **include both study patient number and kit ID in each sample collection form.**
- Be careful to choose the kit bag for the **correct timepoint**!
- Remember to send the remaining materials in the bag for the timepoint (cryovials and labels) to the laboratory together with the sample collection form and the samples.
- Please note that the samples should be sent to the laboratory at room temperature in an **upright position**. If needed, a pneumatic tube system may be used for transportation.
- Unused materials must **not** be used for another trial patient and the kit should be discarded after the last timepoint.

2.4. Data entry

The laboratory will retain the sample collection forms and the study coordinator should collect them at regular intervals for data entry purposes. In the beginning of the study, please collect the forms after the first 2-3 patients and check that they have been completed correctly. If not, please follow up as appropriate by the ICU staff and laboratory.

3. INSTRUCTIONS FOR THE LABORATORY

The samples will usually be processed by personnel at the local hospital laboratory but could, if agreed with the sponsor, be processed by the study personnel at the ICU.

3.1 Materials for sample processing

The laboratory will receive blood samples from the ICU four times/patient, or less if a patient is discontinued from the trial. At each timepoint the samples will be sent with a sample collection form and a bag with cryovials and labels for the cryovials. Boxes for storage of the frozen samples will be provided by the study coordinator as needed.

Figure 2 presents the information included on the labels for cryovials. For example, the label for the first serum aliquot of the 72 h time point would be 12345678-SC-VAR-BLD-**SER-13**. The laboratory should be careful to choose the labels for the correct **sample type, PLA or SER**.

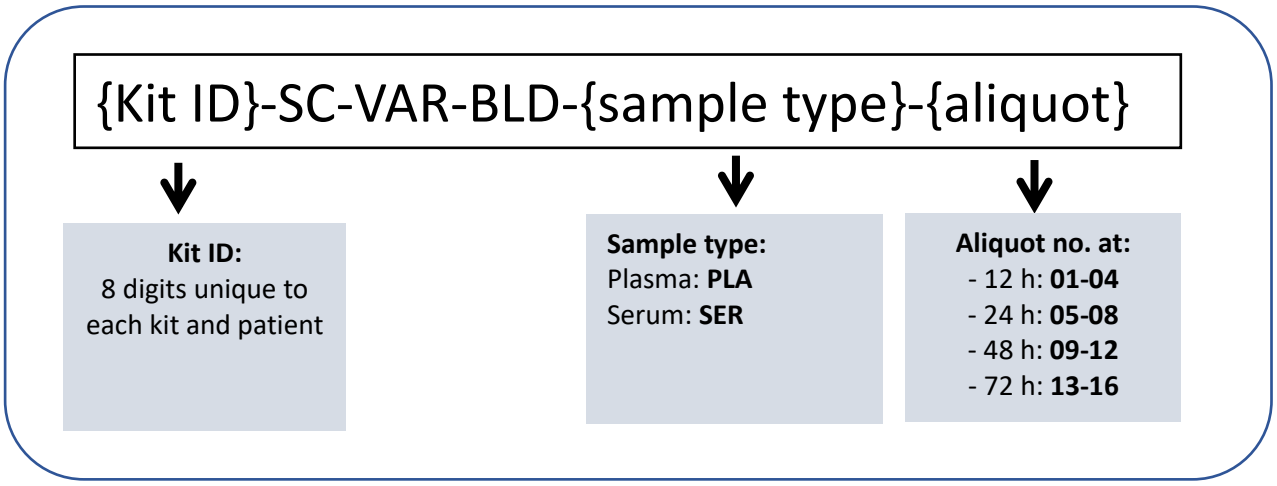

Figure 2. Information included on the labels for cryovials. SC is the acronym for STEPCARE.

3.2. How to apply the labels

The labels are designed to stick onto clean and dry cryovials at ambient temperature. Use the method described in Figure 3 below.

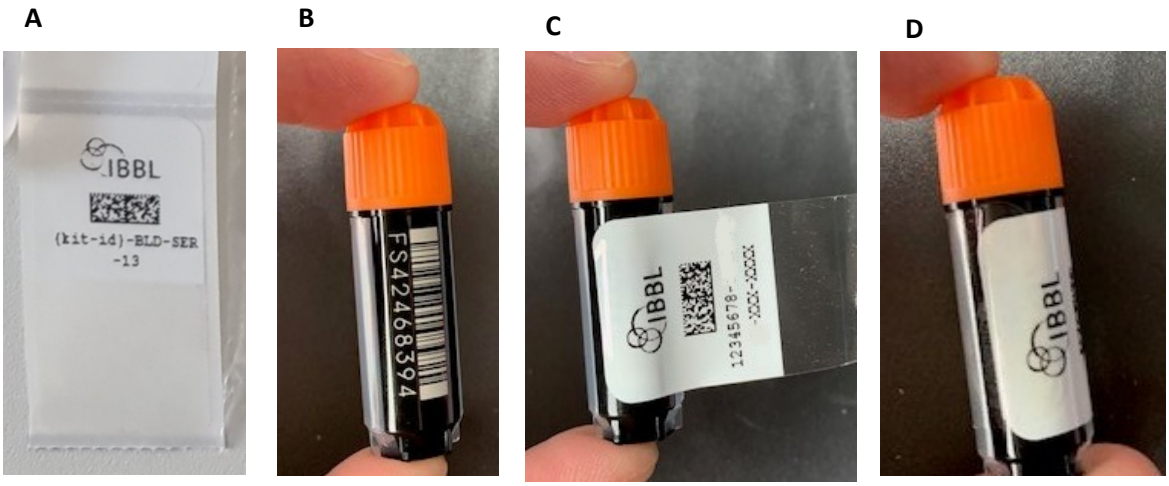

Figure 3 A-D. Steps for how to apply the labels.

A. Pull on the support to peel off the label. Be careful not to touch the sticky part of the label.
B. Hide the barcode on the cryovial with the label.
C. Place the white part of the label on the tube as shown in the picture.
D. Roll the label firmly around the cryovial to ensure good contact and adherence. The transparent part of the label will go over the white part as a second layer. Be sure that there are no air bubbles between the label and the cryovial.

3.3. Sample processing instructions

Use the cryovials, labels, and the form provided together with the blood collection tubes. **Please be careful to choose the labels for the right sample type, PLA or SER.**

Plasma (at 12, 24, 48, and 72 h)

1. Centrifuge the EDTA tube as soon as possible at 2000 g for 10 min at room temperature (preferably slow deceleration at the end with ~50% brake force).
2. Label 4 cryovials with the labels for plasma, ########-**PLA**-{aliquot no.}
3. Using a pipette, aliquot 500 µL of plasma into each of the cryovials labelled with **PLA**. Fill 500 µL in the cryovial with the **lowest aliquot number** before continuing to the next vial. Split any remaining plasma evenly in the vials.
4. **At 12 h only, save the EDTA tube** after removing the plasma. Discard the tube for the other timepoints.
5. As soon as possible put the samples, including the EDTA tube at 12 h, in long term storage at -80°C in the provided boxes
6. Complete the sample collection form.

Serum (at 12, 24, 48, and 72 h)

1. Allow the blood in the SST tube to clot at room temperature in an upright position for at least 45 min after sampling. Ensure that the sample is clotted before centrifugation. Leave the tube no longer than 2 h.
2. Centrifuge the SST tube at 2000 g for 10 min at room temperature (preferably slow deceleration at the end with ~50% brake force).
3. Label 4 cryovials with the labels for serum, ########-**SER**-{aliquot no.}
4. Using a pipette, aliquot 500 µL of serum into each of the cryovials labelled with **SER**. Fill 500 µL in the cryovial with the **lowest aliquot number** before continuing to the next vial. Split any remaining serum evenly in the vials.
5. As soon as possible put the samples in long term storage at -80°C in the provided boxes
6. Complete the sample collection form.

PAXgene RNA tube (at 48 h)

1. Leave the pre-labelled PAXgene RNA tube upright at room temperature for a minimum of 2 h and a maximum of 24 h after sample collection.
2. Place the tube in long term storage at -20°C.
3. Complete the sample collection form.

3.4 Sample storage and registration

The samples should be placed in the freezer in the provided boxes, preferably in the order they were collected and without leaving empty spaces.

The boxes with the cryovials should be stored at -80°C and the PAXgene RNA tubes at -20°C in freezers at the hospital. Temperature logs must be kept for all the freezers storing samples.

The barcode of the labels on the vials should be scanned in batches during the trial and an electronic file compiled including sample IDs and box numbers. The file of the samples in storage should be sent to the study coordinator at least once in the beginning of the trial, preferably after 10-20 patients, and before any sample shipments.

Sites that prefer to continuously record samples in storage instead of scanning samples in batches will receive an alternative version of the sample collection form from the sponsor.

4. SHIPMENT

Samples should be shipped to IBBL in Luxembourg at the end of the trial, or if necessary, at regular intervals (not more than every 6 months to avoid unnecessary shipping costs). The shipment will be organized by the sponsor in collaboration with the study coordinator at the site. The sponsor will pay for the shipment costs. Detailed instructions will be provided before shipment.

5. CONTACT

Contacts for laboratory or biomarker questions:
helena.levin@med.lu.se
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6. VERSION HISTORY

Version 1.0, September 19th 2023
Version 1.1, November 21st 2023: "Split any remaining plasma/serum evenly in the vials" was added on page 7 to plasma step 3 and serum step 4.

SAMPLE COLLECTION FORM
for ICU Staff

Patient study number:

Patient's kit ID:

Kit ID-label

########-SC-VAR

Do not use the partly transparent labels!

Contact at the ICU:
Name <complete>,
Phone <complete>, Email <complete>

Material for one patient:
1 kit with a unique kit ID in all labels
4 bags (one/time point) with collection tubes and lab material
4 sample collection forms (one/time point)
A strip of kit ID labels for forms

1. **Complete the red square above.**

2. **Fill out:**

a. Time point (hours after randomisation):

12 h ☐ 24 h ☐ 48 h ☐ 72 h ☐

Draw samples as close as possible to the designated time point (+/- 8 hours are allowed)

b. Sampling date and time:

D D M M M Y Y Y Y H H : M M

Record month with 3 letters and time in 24-hour format, e.g. 15APR2023 15:30

c. Sampling performed by (name/initials):

3. **Use the bag for the correct time point and draw the samples in the following order.**
   - **1 x SST tube (yellow cap)**
   - **1 x EDTA tube (purple cap)**
   - **At 48 h: 1 x PAXgene RNA tube** – hold upright to prevent back-flow

4. **Invert the SST tube (yellow) 5-6 times. Invert the EDTA tube (purple) and PAX tube 8-10 times.**

5. **Send to the laboratory:**
   - this form +
   - remaining material in the bag of the time point +
   - samples in an upright position

Record the patient´s kit ID in the medical record. The kit must **not** be used for another patient.
After the last time point (72 h), discard any unused material.

SAMPLE COLLECTION FORM
for the laboratory

1. **Check on the back of this form that:**
   - the **red square** has been fully completed.
   - the **time point** corresponds to the aliquot numbers at the end of the labels, i.e. **12 h;** 01-04, **24 h;** 05-08, **48 h;** 09-12, **72 h;** 13-16.

2. **Handle the samples according to the provided instructions.**

3. **Complete the information below.** Record date and time in the correct format (e.g. 15APR2023 15:30 or tick if same as for plasma.)

Plasma **Date and time placed in freezer:** D D M M M Y Y Y Y H H : M M	**At 12 h: EDTA-tube** **Date and time placed in freezer:** D D M M M Y Y Y Y H H : M M ☐ same as for plasma
Serum **Date and time placed in freezer:** D D M M M Y Y Y Y H H : M M ☐ same as for plasma	**At 48 h: PAXgene RNA tube** **Date and time placed in freezer:** D D M M M Y Y Y Y H H : M M
Samples handled by (name/initials):	

If any deviations occurred, please specify:

Save this form! The forms will be collected regularly by the study coordinator.