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ESMI

European Spinocerebellar Ataxia Type 3/Machado-Joseph Disease Initiative

Manual for Biosample collection

Version 3, August 2020

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CONTENT

1. INTRODUCTORY NOTES / Changes compared to version 2

- The first version of this manual was written in June 2016 to give guidance to laboratory technicians/researchers regarding biosample collection and processing procedures to be executed related to work package 4 (biochemical markers) of the JPND ESMI project.

- A revised second version was implemented in December 2016 with the aim to improve standardization of the procedures and account for specificities of individual research centers.

- The JPND funding period of ESMI finished in Spring 2020. The established cohort will be maintained and related activities continued to the extent possible, based on other funding. For this purpose, the biosample collection manual has been adapted, making use of experiences gained over the past years, and aimed at collecting sufficient biomaterial for ongoing and future projects at a reasonable cost.

- Major alterations compared to the previous (second) version of this manual are the following:

1) Number of tubes

PaxGene (RNA): 2 tubes *(previously 4 tubes)*
CPT (PBMC): 2 tubes *(unchanged)*
SST (Serum): 3 tubes *(previously 2 tubes)*
PPT (Plasma): 2 tubes *(previously 1 tube)*
EDTA (Whole blood): 1 tube *(unchanged)*

2) Volume of aliquots

500 µl *(previously 1000 µl)*

3) All the biomaterial collected should be stored.

While the previous version included the optional suggestion to discard remaining low volumes of samples, in this version partners are requested to store all collected materials (even small volumes) to the extent that local conditions allow.

4) Additional washing step during PBMC collection

An additional washing step has been introduced in the PBMC isolation procedure in order to obtain a higher yield.

5) Report in the SCA registry database

Information regarding sample processing and number and volume of aliquots should be reported in the SCA Registry database.

6) Participating research centers

Information regarding participating centers was updated.

2. STUDY INFORMATION AND DESCRIPTION

2.1 Purpose

Spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3) is the most common familial ataxia. Although the gene mutation causing SCA3 is known, currently there is no treatment available for the disease. However, as there is an advanced understanding of the mechanisms underlying SCA3, new therapeutic approaches are being developed. To enable future interventional trials in SCA3, availability of a large trial ready cohort is mandatory. The European Spinocerebellar Ataxia Type3/Machado Joseph Disease Initiative (ESMI) has built up a large cohort of symptomatic and pre-symptomatic SCA3 mutation carriers as well as healthy controls. Development and validation of innovative disease biomarkers has been a major focus of ESMI.

Probably the most critical step in biomarkers research field is the collection and processing of biosamples. Laboratory tests can be repeated, but if the biosample itself is not correctly drawn, labeled and processed, results may not be accurate even if the laboratory assays are precise. It is therefore important that variation in measurement values reflect true differences between the study samples rather than differences in biosampling collection and processing procedures.

Taking these concerns into account, this manual describes appropriate guidance to clinical research personnel in order to obtain high quality biosamples intended for use in biomarkers clinical research in the framework of the ESMI network.

2.2 Participating research centers and groups

Site	Institution	PI (name)	PI (email)
Aachen, Germany	University Hospital Aachen	Kathrin Reetz	kreetz@ukaachen.de
Azores, Portugal	Fundação Gaspar Frutuoso (FGF), University of Azores	Manuela Lima	maria.mm.lima@uac.pt
Bonn, Germany	German Center for Neurodegenerative Diseases (DZNE)	Thomas Klockgether	thomas.klockgether@ukbonn.de
Coimbra, Portugal	Center for Neuroscience and Cell Biology (CNC), University of Coimbra	Luis P. Almeida	luispa@cnc.uc.pt
Essen, Germany	University Hospital Essen	Dagmar Timmann	Dagmar.Timmann-Braun@uni-duisburg-essen.de
Groningen, Netherlands	University of Groningen	Jeroen de Vries	j.j.de.vries01@umcg.nl
Heidelberg, Germany	University Hospital Heidelberg	Heike Jacobi	Heike.Jacobi@med.uni-heidelberg.de
London, UK	University College London	Paola Giunti	p.giunti@ucl.ac.uk
Nijmegen, Netherlands	Radboud University Medical Center (RUMC)	Bart van de Warrenburg	B.vandeWarrenburg@neuro.umcn.nl
Santander, Spain	Fundación Instituto de Investigación Marqués de Valdecilla	Jon Infante	jinfante@humv.es
Tübingen, Germany	University of Tübingen	Ludger Schöls Olaf Riess	ludger.schoels@uni-tuebingen.de Olaf.Riess@med.uni-tuebingen.de

2.3 Responsibilities

Each research center is responsible for providing the appropriate recommendations and training to their local research personnel and technicians, according to the guidelines described in this manual. Each partner is also responsible for organizing the documentation (including Ethical Committee approval and written informed consent).

Participation in this study is voluntary. Participants should be informed and sign the informed consent prior to sample collection.

3. PREPARATION OF BIOSAMPLE COLLECTION

3.1 Participants and visits

Samples will be collected at a baseline visit (1) and subsequent annual follow-up visits (2, 3 and so on). If samples cannot be collected at the same time of clinical evaluation, they should be collected within one month, if possible.

3.2 Sample description

Biosamples to be collected in this study are described in Table 1.

Table 1. Sample description.

Sample		Collection tube		Number x Volume	Purpose	Baseline visit	Follow up visit(s)
Blood	**Whole blood**	#1 #2	PAXgene RNA	2 x 2.5 mL	RNA (RNAseq/qPCR)	X	X
	PBMC	#5 #6	Cell Preparation Tube (CPT)	2 x 8.0 mL	Protein RNA	X	X
	Serum	#7 #8 #12	Serum Separator Tube (SST)	3 x 8.5 mL	Exosomes (Protein/RNA) Protein	X	X
	Plasma	#9 #13	Plasma Preparation Tube (PPT)	2 x 8.5 mL	Exosomes (RNA) Protein	X	X
	Whole blood	#10	EDTA Tube	1 x 4.0 mL	DNA	X	Optional
Cerebrospinal Fluid		#11	Polypropylene Tube	1 x 15 mL	qPCR Protein Exosomes	X	X
Fibroblasts		#14	Polypropylene Tube	Small biopsy	iPSC	One time during study course	

3.3 Collection tubes

Collection tubes were carefully selected to suit the need of this study. Do not replace or supplement any of the tubes with your own supplies unless you have received approval to do so. A description of the different collection tubes and appropriate storage conditions before

sample collection is presented in Table 2. More detailed information about each tube can be found in the Becton Dickson supplier's website http://www.bd.com/vacutainer/.

Table 2. Collection tubes description.

Sample			Collection tube	Reference	Cap color	Additives	Storage
Blood	Whole blood	#1 #2	PAXgene RNA	BD #762165		RNA stabilizing agent	18-25°C
	PBMC	#5 #6	Cell Preparation Tube (CPT)	BD #362780		Sodium Heparin/Ficoll	18-25°C
	Serum Plasma	#7 #8 #12	Serum Separator Tube (SST)	BD #367953		Clot Activator/ Gel	4-25°C
		#9 #13	Plasma Preparation Tube (PPT)	BD #362799		K2EDTA /Gel	4-25°C
	Whole blood	#10	EDTA Tube	BD #367839		K2EDTA	4-25°C
Cerebrospinal Fluid		#11	Polypropylene Tube	BD #352096 (or equivalent)		No additives	-
Fibroblasts		#12	Polypropylene Tube	BD #352096 (or equivalent)		No additives	-

3.4 Sample labelling

3.4.1 Collection tubes labelling

Each collection tube should be unequivocally labelled prior to blood collection.

Each center will use their local labeling system, but efforts should be done to avoid hand writing the label to avoid misspelling words. Labels should be printed and pasted on the tubes.

The following advisements should be followed to label the tubes:

- Confirm patient identity just before blood collection
- Place label vertical on the tube
- Avoid misaligning the label
- Ensure the label has completely adhered to the tube.

An example of tubes labeling can be found in Annex 1.

3.4.2 Aliquot labeling

Cryovials will be used to store PBMCs and aliquots of plasma/serum/whole blood after blood sample processing.

Each cryovial should be unequivocally labelled before blood processing.

Each center will use their local labeling system, but efforts should be done to avoid hand writing in the labeling to avoid misspelling words.

Information about each aliquot might be written in the back of the Sample Identification Form (see section 2.2).

3.4.3 General ESMI biosample code

Samples to be exchanged between centers need to be identified by a **general ESMI biosample code**, generated as following:

ESMI Patient ID / Nº of visit; Nº of original blood tube; Nº of the aliquot

*For example, the sample with the **ESMI biosample code 3475490/172** correspond the aliquot nº2 original from the tube #7 (i.e. Serum), collected at the baseline (1), from the patient 3475490.*

A list of ESMI biosample codes attributable to the different blood samples can be found in Annex 2.

Every time a sample is transferred between centers it should be accompanied by a Biosample Sheet (Annex 3) that links the ESMI biosample code to the local biosample code/labeling of the shipped aliquot.

3.5 Sample identification forms

The sample identification forms present in Annex 4 (Blood Sample Identification Form), 5 (CSF Sample Identification Form) and 6 (Fibroblasts Sample Identification Form) should be filled each time biosampling collection is conducted. Sample identification forms should be properly archived at each center; the information contained in the form and considered as most relevant **should be reported in the SCA Registry database**.

The sample identification forms contain the following information:

- Research center
- Number of the visit
- Date of collection
- Time of collection
- ESMI identification number of the sample/patient
- Time of last meal
- Number of tubes collected
- Number of aliquots
- Volume of incomplete aliquots
- Any occurrence during collection/processing

Additionally, the back of the Sample Identification Form might be filled with any relevant information/observations occurring during sample collection and processing.

3.6 Material and equipment

3.6.1 Material

Collection tubes and any other consumables and/or reagents needed for biosample collection/processing/storage will be provided by each research center. Each center is responsible to ensure that collection blood kits are compatible with the tubes described in section 2.3.

A list of the material necessary for blood collection and processing can be found in Annex 7.

3.6.2 Equipment

In order to collect and process samples consistently across all centers and ensure the highest quality samples possible, sites need to have access to the following equipment:

- Swing-out rotor type centrifuge at room temperature.
- -20ºC freezer.
- -80ºC freezer.

4. COLLECTION OF BLOOD BIOSAMPLES

4.1 Participants preparation

Efforts should be made to ensure the procedure is easy and as painless as possible. Clinicians/technicians/study nurses should remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The ESMI study collects approximately 67.5 mL of blood from each participant. The technician should reassure any participant who is concerned about the volume of blood collected that the total amount drawn is only about 6 – 7 tablespoons, although it may look more. The technician may also assure participants that people donate almost 10 times as much blood (450 mL) when they donate blood.

4.2 Time for blood collection

If possible, blood samples should be collected during morning between 8:00 and 12:00, preferably fasting. The time of blood collection and the time of last meal is registered in the sample identification form and should be recorded in the SCA Registry database.

4.3 Venipuncture procedure

The blood collection should be performed according to each center recommended procedure for standard venipuncture technique. The following general recommendations should be followed:

- Assess participant disposition.
- Confirm participant identity.
- Place donor's arm in a downward position.
  Hold collection tubes in a vertical position, below the donor's arm during blood collection.
  CRITICAL POINT: Make sure tube additives do not touch stopper or end of the needle during venipuncture.
- Release tourniquet as soon as blood starts to flow into tube.
- Make sure the tubes are totally filled.
- **Immediately invert the tube, respecting the number of inversions of each tube.**

See Annex 8 for a workflow of venipuncture procedure.

4.4 Order of tube collection

Technicians should be familiarized with the different tubes and a blood collection tray with the tubes in the correct order should be prepared in advance.

To guarantee the acquisition of all the different sample types, tubes should be filled with blood in the following order:

#1.	PAX Gene RNA Tube	2.5 mL
#5.	Cell Preparation Tube	8.0 mL
#7.	Serum Separator Tube	8.5 mL
#9.	Plasma Preparation Tube	8.5 mL
#10.	Plasma EDTA Tube	4.0 mL
#13.	Plasma Preparation Tube	8.5 mL
#8.	Serum Separator Tube	8.5 mL
#2.	PAX Gene RNA Tube	2.7 mL
#6.	Cell Preparation Tube	8.0 mL
#12.	Serum Separator Tube	8.5 mL

A representative picture of blood collection tubes, in this specific order, can be found in Annex 9.

The number of collected tubes and the number and volume of aliquots should be registered in the SCA Registry database.

Specific instructions for storage and use of each tube should be taken into account and are described in the following sections.

4.5 Number of tube inversions

Immediately after blood collection tubes should be immediately mixed by complete inversion according to table 3.

The number of inversions of each specific tube has to be respected.

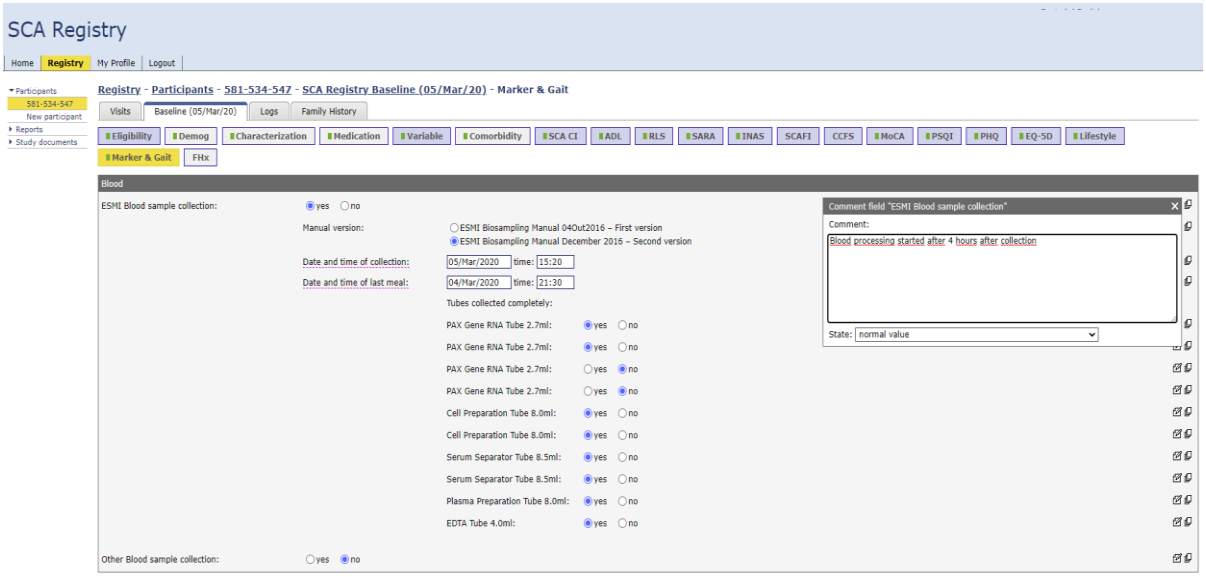
Tubes should then be stored upright, at room temperature, until processing (see section 4).

Table 3. Number of tube inversions after blood collection

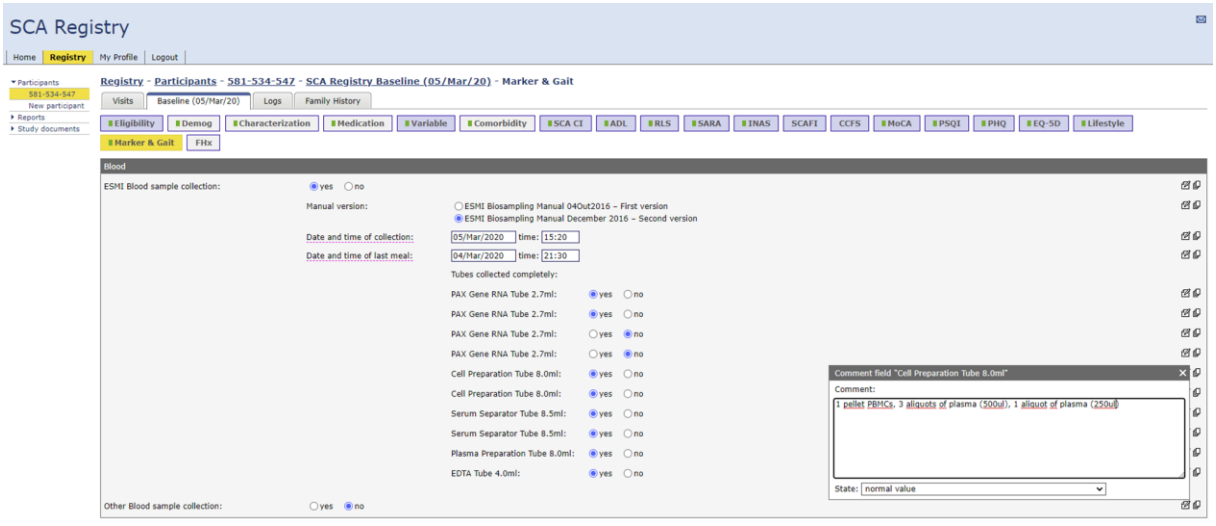
Number	Collection tube	Cap	Number of inversions
#1 **#2**	PAXgene RNA		8-10 times
#5 **#6**	Cell Preparation Tube (CPT)		8-10 times
#7 **#8** **#12**	Serum Separator Tube (SST)		5 times
#9 **#13**	Plasma Preparation Tube (PPT)		8-10 times
#10	Plasma EDTA		8-10 times

4.6 Reporting

The sample identification should be filled immediately after blood collection and processing. **Any deviation from the instructions contained in this manual or any occurrence during collection and processing** (ex: hemolysis during collection, timeframe for blood processing not respected, sample contamination, etc) **should be reported in the SCA Registry database** in a "comment field" (see example below).



CRITICAL: The number and volume of aliquots obtained from each tube should be registered. This information should also be reported in the SCA Registry database in "comment field" corresponding to each collection tube (see example below).



5. PROCESSING OF BLOOD BIOSAMPLES

The blood processing should start as soon as possible and within a timeframe of 2 hours after blood collection to ensure high quality of samples.

If sample processing starts after the period of 2 hours, it should be reported in sample identification form and in the SCA Registry database.

Specific instructions for blood processing for each blood collection tube are described in the following sections.

5.1 PAXGene Tubes (#1 and #2)

- Incubate tubes upright at room temperature for at least 2 hours and no more than 6 hours.
- Freeze tubes at -20ºC for 24 to 72 hours.
- Store tubes at -80ºC.

For more specifications about PAXGene Tubes, please find the BD product insert at: http://www.bdbiosciences.com/ds/ab/others/PAXgene_Blood_RNA_Tube_Product_Circular.pdf.

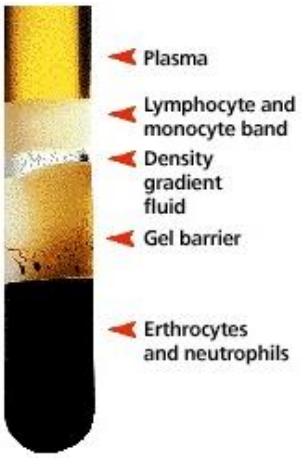
5.2 Cell Preparation Tubes (CPT) (#5 and #6)

- Centrifuge tube at room temperature (18-25˚ C) in a balanced, swing-out rotor type, for 30 minutes at 1700 RCF, with the brake off.
- After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see image).

For plasma collection:

Aspirate the plasma without disturbing the cell layer plasma and divide it into 500 µL cryovials aliquots. Freeze immediately at -80ºC.

Critical note: No plasma should be discarded. Any remaining volume should be kept, even if resulting in aliquots less than 500 µL, and the volume should be registered in the SCA Registry database (see section 3.6). In case of space constrains, the volume of the aliquots can be increased up to 1000 µL.



For PBMC collection:

- Collect cell layer with a sterile Pasteur Pipette and transfer to a 15 mL polypropylene tube.
- Refill blood collection tube with 3 ml PBS and remove cells from the gradient layer by up and down pipetting
- Collect PBS/ PBMCs with a sterile Pasteur Pipette and transfer to the polypropylene tube from step 1
- Wash cells with sterile Phosphate Buffered Saline (PBS), without magnesium and calcium, by adding PBS up to the 15 mL mark.
- Centrifuge for 15 minutes at 300 RCF, at room temperature, with the brake on.

- Discard the supernatant.
- Repeat the washing step:
  - resuspend the PBMCs pellet in 3 mL of PBS;
  - add PBS up to the 15 mL mark;
  - centrifuge 15 minutes at 300 RCF, at room temperature with the brake on.
- At this step, choose one of the options:

  Option 1 (if microcentrifuge available; to optimize storage space):
  - discard the supernatant;
  - resuspend the PBMCs pellet in 500 µL of PBS;
  - transfer to a cryovial and centrifuge at 300 RCF, at room temperature, with the brake on;
  - discard the supernatant removing all the PBS from the cell pellet;
  - freeze cryovials with cell pellet at -80ºC.

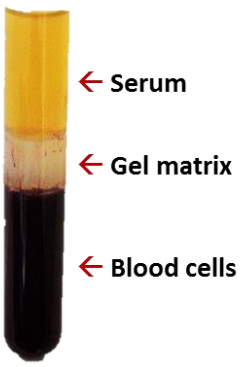
  Option 2:
  - Discard the supernatant removing all the PBS from the cell pellet;
  - Freeze 15 mL propylene tube with cell pellet at -80ºC.

For more specifications about CPT Tubes, please find the BD product insert at: http://www.bdbiosciences.com/ds/ab/others/PI_PPT_March_2016_VDP40162-03_Web.pdf

5.3 Serum Separator Tubes (SST) (#7, #8 and #12)

- Allow blood to clot in a vertical position for a minimum of 30 minutes and not more than 2 hours.
- Centrifuge the tube in a balanced, swing-out rotor type centrifuge at room temperature at 1,100 RCF for 10 minutes, with the brake off.
- Remove the BD Hemogard™ Closure.
- Aspirate the serum layer into a 10 mL syringe using a 16G needle (see image). Alternatively, a sterile Pasteur pipette can be used to transfer serum into the 10 mL syringe.
- Replace the needle with a 0.8 micron filter and push the serum through the filter drop by drop into a 15 mL polypropylene tube.

  NOTE: When aspirating into the syringe/Pasteur pipette, be sure not to disturb the red cell layer/buffy coat with the tip of the needle.
- Divide serum into 500 µL cryovials aliquots and freeze immediately at -80ºC.



Critical note: No plasma should be discarded. Any remaining volume should be kept, even if resulting in aliquots less than 500 µL, and the volume should be registered in the SCA Registry database. In case of space constrains, the volume of the aliquots can be increased up to 1000 µL.

5.4 Plasma Preparation Tube (PPT) (#9 and #13)

- Centrifuge tube in a balanced, swing-out rotor type centrifuge at room temperature at 1,100 RCF for 10 minutes, with the brake off.
- Remove the BD Hemogard™ Closure.
- Aspirate the plasma layer into a 10 mL syringe using a 16G needle. Alternatively, a sterile Pasteur pipette can be used to transfer plasma into the 10 mL syringe.

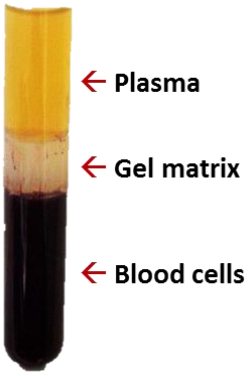
- Replace the needle with a 0.8 micron filter and push the plasma through the filter drop by drop into a 15 mL falcon tube.

  Note: When aspirating into the syringe/Pasteur pipette, be sure NOT to disturb the red cell layer/buffy coat with the tip of the needle.

- Divide plasma into 500 µL cryovials aliquots and freeze immediately at -80ºC.

Critical note: No plasma should be discarded. Any remaining volume should be kept, even if resulting in aliquots less than 500 µL, and the volume should be registered in the SCA Registry database (see section 3.6). In case of space constrains, the volume of the aliquots can be increased up to 1000 µL.

For more specifications about PPT Tubes, please find the BD product insert at: http://www.bdbiosciences.com/ds/ab/others/PI_PPT_March_2016_VDP40162-03_Web.pdf



5.5 Plasma EDTA Tube (#10)

- Divide whole blood into 500 µL cryovials aliquots.

  Critical note: No blood should be discarded. Any remaining volume should be kept, even if resulting in aliquots less than 500 µL, and the volume should be registered in the SCA Registry database (see section 3.6).

  In case of space constrains, the volume of the aliquots can be increased up to 1000 µL.

- Freeze directly at -80ºC.

An overview table for blood collection and processing can be found in Annex 10.

6. COLLECTION AND PROCESSING OF CSF BIOSAMPLES

6.1 Participants preparation

Each participant should be informed about the importance of the procedure in the context of the study. Potential complications and risks should be discussed with the clinicians. Each participant must sign the informed consent.

Clinicians and technicians should do efforts to optimize patient comfort and minimize risks of adverse events.

CSF should be collected in the morning, preferably fasting. Record time of last meal in CSF Sample Identification Form.

6.2 Lumbar puncture and CSF collection

The lumbar puncture should be performed according to each center recommended standard operating procedure, by trained personnel.
The following recommendations should be followed:

- Confirm participant identity.

- Assess participant disposition.
- Confirm participant identity.
- Ensure antiseptic cleansing and anesthesia.
- In case of bleeding at the puncture site, discard the first 1mL of CSF and record the event in CSF Sample Identification Form.
- Collect between 12-15mL of CSF to a polypropylene tube.
- Mix the CSF gently by turning the tube upside down for 3-4 times (cap on).

6.3 CSF sample processing

The CSF processing should start as soon as possible and within a timeframe of 2 hours after blood collection to ensure high quality of samples.

If sample processing starts after the period of 2 hours, it should be reported in the SCA Registry database (see section 5.4).

Specific instructions for CSF processing are the following:

- Centrifuge the tube in a balanced, swing-out rotor type centrifuge at room temperature at 1,100 RCF for 10 minutes.
- Aspirate the CSF and divide the fluid into 500 µL cryovials aliquots.

  Critical note: No CSF may be discarded. Any remaining volume should be kept, even if resulting in aliquots less than 500 µL, and the volume should be registered in the SCA Registry database (see section 5.4). In case of space constrains, the volume of the aliquots can be increased up to 1000 µL.
- Freeze immediately at -80ºC.

6.4 Reporting

The CSF Sample Identification Form should be filled immediately after CSF collection and processing. **Any deviation from this manual or any occurrence during collection and processing should be reported in the SCA registry database (see below).**

7. COLLECTION OF FIBROBLAST SAMPLES

Skin biopsies should be performed according to the local procedures of the respective center.

The fibroblasts sample identification (Annex 6) should be filled immediately after fibroblast collection and processing.

The protocol used should be described in the back of the Fibroblasts Sample Identification Form. This information will only be shared between centers, if necessary.

8. STORAGE AND EXCHANGE OF BIOSAMPLES

Storage of biomaterials will be done locally in appropriate cooling devices at -80ºC, after appropriate processing.

The clinical SCA Registry database contains information about available samples, their storage site and the clinical data associated with the samples. Information on individual aliquots currently needs to be registered in the "comment" field but will prospectively be included in a specific aliquot report field.

Instructions for biosample exchange between centers have been established by the ESMI Executive Board and the current version can be found on the ESMI internal web space or can be requested to the ESMI biomaterial coordinator.

9. SAMPLE COLLECTION AT EACH RESEARCH CENTER

Specific alterations to the protocol have to be performed by some research centers in order to adapt biosampling collection to local conditions, without compromising biosampling quality. These alterations are summarized in table 4.

Table 4. Biosampling collection specificities for each center

Research Center	Contact Person(s)	Tubes collected	Processing	Storage
Aachen, Germany: UKA	• Kathrin Reetz kreetz@ukaachen.de	All tubes	According to manual	DZNE, Bonn, Germany
Azores, Portugal: FGF	• Mafalda Raposo mafalda.sb.raposo@uac.pt • Manuela Lima maria.mm.lima@uac.pt	All tubes (with limitations: On Flores and Graciosa Islands, CPT tubes are not collected.)	In São Miguel, according to the manual. In Flores, Graciosa and Terceira Islands, Paxgene tubes will be kept at 4˚C and aliquots from PPT, SST, EDTA and CPT (if collected) will be kept at -20˚C for 24h/48h before -80ºC freezing.	Local
Bonn, Germany: DZNE	• Jennifer Faber Jennifer.Faber@dzne.de	All tubes	According to manual	Local
Coimbra, Portugal: CNC	• Magda Santana mmsantana@uc.pt	All tubes	According to manual	Local
Essen, Germany: UKE	• Dagmar Timmann Dagmar.Timmann-Braun@uni-duisburg-essen.de	Only PaxGene tubes	Tubes will be sent to Bonn in 24h at 4ºC; once arrived, processing will be performed according to manual.	DZNE, Bonn, Germany
Groningen, Netherlands: UMCG,	• Jeroen de Vries j.j.de.vries01@umcg.nl	All tubes	According to manual	Local
Heidelberg, Germany: Univ Hosp HD	• Heike Jacobi Heike.Jacobi@med.uni-heidelberg.de	All tubes	According to manual	DZNE, Bonn, Germany
London, UK: UCL	• Paola Giunti p.giunti@ucl.ac.uk • Hector Garcia-Moreno h.garcia-moreno@ucl.ac.uk	All tubes	According to manual	Local (with limitations)
Nijmegen, Netherlands: RU	• Judith VanGaalen Judith.vanGaalen@radboudumc.nl	PaxGene tubes, SST, PPT, EDTA	According to manual, except for SST and PPT (without filtration step)	Local
Santander, Spain:	• Jon Infante	All tubes	According to manual	CNC,

UHMV	jinfante@humv.es			Coimbra, Portugal
Tübingen, Germany UT	• Ludger Schöls ludger.schoels@uni-tuebingen.de • Holger Hengel holger.hengel@uni-tuebingen.de	All tubes	According to manual	Local

For any questions please contact:

Magda Santana

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or

Ruth Herberz

ruth.herberz@dzne.de

10. ANNEXES

1. **Collection tubes labelling**
2. **ESMI biosamples codes**
3. **Biosample sheet**
4. **Blood sample identification form**
5. **CSF sample identification form**
6. **Fibroblasts sample identification form**
7. **Material and reagents**
8. **Blood collection venipuncture procedure**
9. **Blood collection tubes per patient**
10. **Overview table: Blood collection and processing**

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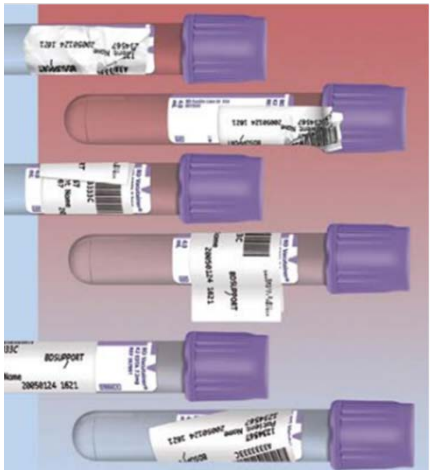
Collection tubes labeling

INCORRECT	CORRECT
	

GENERAL ESMI BIOSAMPLE CODES

ESMI Patient ID / Nº of visit; Nº of original blood tube; Nº of the aliquot

Note: For CPT, PBMC will correspond to aliquot 0.

***Example: ESMI PATIENT ID:* 3475490** *(hypothetical patient)*

Visit	Tube		Aliquot	ESMI biosample code (Tube)	ESMI biosample code (Aliquot)
1 (Baseline)	PaxGene	#1	N/A	3475490/11	N/A
		#2	N/A	3475490/12	N/A
		#3	N/A	3475490/13	N/A
		#4	N/A	3475490/14	N/A
	CPT	#5	PBMCs Plasma aliquot 1 Plasma aliquot 2 (…)	3475490/15 3475490/15 3475490/15 (…)	3475490/150 3475490/151 3475490/152 (…)
		#6	PBMCs Plasma aliquot 1 Plasma aliquot 2 (…)	3475490/16 3475490/16 3475490/16 (…)	3475490/160 3475490/161 3475490/162 (…)
	SST	#7	Serum aliquot 1 Serum aliquot 2 (…)	3475490/17 3475490/17 (…)	3475490/171 3475490/172 (…)
		#8	Serum aliquot 1 Serum aliquot 2 (…)	3475490/18 3475490/18 (…)	3475490/181 3475490/182 (…)
		#12	Serum aliquot 1 Serum aliquot 2 (…)	3475490/112 3475490/112 (…)	3475490/1121 3475490/1122 (…)
	PPT	#9	Plasma aliquot 1 Plasma aliquot 2 (…)	3475490/19 3475490/19 (…)	3475490/191 3475490/192 (…)
		#13	Plasma aliquot 1 Plasma aliquot 2 (…)	3475490/113 3475490/113 (…)	3475490/1131 3475490/1132 (…)
	EDTA	#10	Aliquot 1 Aliquot 2 (…)	3475490/110 3475490/110 (…)	3475490/1101 3475490/1102 (…)
	CSF	#11	Aliquot 1 Aliquot 2 (…)	3475490/111 3475490/111 (…)	3475490/1111 3475490/1112 (…)
2 (One-year follow up)	PaxGene	#1	N/A	3475490/21	N/A
		#2	N/A	3475490/22	N/A
		#3	N/A	3475490/23	N/A
		#4	N/A	3475490/24	N/A
	CPT	#5	PBMCs Plasma aliquot 1 Plasma aliquot 2 (…)	3475490/25 3475490/25 3475490/25 (…)	3475490/250 3475490/251 3475490/252 (…)
		#6	PBMCs Plasma aliquot 1 Plasma aliquot 2 (…)	3475490/26 3475490/26 3475490/26 (…)	3475490/260 3475490/261 3475490/262 (…)
	SST	#7	Serum aliquot 1 Serum aliquot 2 (…)	3475490/27 3475490/27 (…)	3475490/271 3475490/272 (…)
		#8	Serum aliquot 1 Serum aliquot 2 (…)	3475490/28 3475490/28 (…)	3475490/281 3475490/282 (…)
		#12	Serum aliquot 1 Serum aliquot 2 (…)	3475490/212 3475490/212 (…)	3475490/2121 3475490/2122 (…)
	PPT	#9	Plasma aliquot 1 Plasma aliquot 2 (…)	3475490/29 3475490/29 (…)	3475490/291 3475490/292 (…)
		#13	Plasma aliquot 1 Plasma aliquot 2 (…)	3475490/213 3475490/213 (…)	3475490/2131 3475490/2132 (…)
	EDTA	#10	Aliquot 1 Aliquot 2 (…)	3475490/210 3475490/210 (…)	3475490/2101 3475490/2102 (…)
	CSF	#11	Aliquot 1 Aliquot 2 (…)	3475490/211 3475490/211 (…)	3475490/2111 3475490/2112 (…)

Same principle continues for visits number 3, 4 etc.

ESMI Biosample Sheet

To be filled in by Biosample Coordinator	**Request ID(s)**	
	Provider Institution	
	Recipient Institution	
*To be filled in by **PROVIDER** (before sending material)*	**Date of shipment**	
*To be filled in by **RECIPIENT** (after receiving material)*	**Date of delivery**	

*To be filled in by **Biosample Coordinator***				*To be filled in by **PROVIDER** (before sending material)*				*To be filled in by **RECIPIENT** (after receiving material)*			
Sample	**ESMI Biosample Code**		**Visit date**	**Local biosample code** (only if samples are not labelled with ESMI code)	**Volume at time of shipping [µl]**	**nº of freeze/thaw cycles at time of shipping**	**Observations/Comments**	**Sample contained in shipment? (Yes/No)**	**Remaining volume after experiments [µl]**	**Total nº of freeze/thaw cycles after experiments**	**Observations/Comments**
1	XXX-XXX-XXX	xxx	dd mm yyyy								
2											
3											
4											
5											
(…)											

Notes (by Biosample Coordinator):

Packaging and shipment information (by Biosample Coordinator):

BLOOD SAMPLE IDENTIFICATION FORM

Research center

Label with patient ID (optional)

ESMI Patient Identification Number _____

Date of collection _____ /_____ /_____

Time of collection _____

Number of the visit _____ *(Note: Baseline corresponds to visit 1)*

Time of last meal _____

Collection tubes	Check list	Nº of complete aliquots (=500 µl)	Nº of incomplete aliquots (<500 µl)
1. PAX Gene RNA Tube			
2. PAX Gene RNA Tube			
3. PAX Gene RNA Tube			
4. PAX Gene RNA Tube			
5. Cell Preparation Tube		PBMCs: Plasma:	Plasma: Vol:
6. Cell Preparation Tube		PBMCs: Plasma:	Plasma: Vol:
7. Serum Separator Tube		nº:	nº: Vol:
8. Serum Separator Tube		nº:	nº: Vol:
9. Plasma Preparation Tube		nº:	nº: Vol:
10. Plasma EDTA Tube		nº:	nº: Vol:
13. Serum Separator Tube		nº:	nº: Vol:
14. Plasma Preparation Tube		nº:	nº: Vol:

Notes:

Observations

(ex: volume of incomplete aliquots, copy of label stickers, errors detected that need to be recorded)

CSF SAMPLE IDENTIFICATION FORM

Research center

Label with patient ID (optional)

ESMI Patient Identification Number _____

Date of collection _____ / ____ /____

Time of collection _____

Number of the visit _____ *(Note: Baseline corresponds to visit 1)*

Time of last meal _____

Volume collected _____

Nº of complete aliquots (500 µl)

Nº of incomplete aliquots (<500 µl)

Volume of incomplete aliquots (µl)

Notes:

Observations

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FIBROBLASTS SAMPLE IDENTIFICATION FORM

Research center

Label with patient ID (optional)

ESMI Patient Identification Number _____

Date of collection _____ / _____ /_____

Time of collection _____

Number of the visit _____ *(Note: Baseline corresponds to visit 1)*

Punch site _____

Punch diameter _____ **mm**

Collection Medium _____

Notes:

Observations

Protocol:

Material and reagents

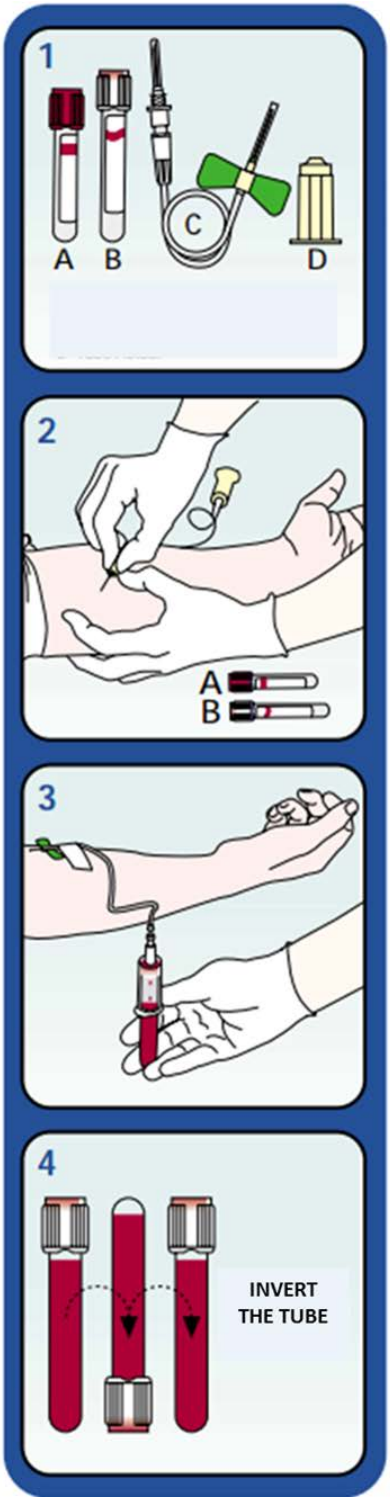
Blood collection

- 1 x 21G butterfly needle
- 1 x Vacutainer needle holder
- 1 x Latex gloves
- 1 x Tourniquet
- Alcohol wipes
- Cotton wool
- Small plasters
- 1 x Sharps bin for used needle or needle/holder combination
- Sample tubes:
    - 2 x Cell Preparation Tube 8.0 mL (BD #362780)
    - 3 x Serum Separator Tube 8.5 mL (BD #367953)
    - 2 x Plasma Preparation Tube 8.5 mL (BD #362799)
    - 1 x Plasma Preparation Tube 4.0 mL (BD #367839)
    - 2 x PAX Gene RNA Tube 2.7 mL (BD #762165)
- Tube labels
- Sample Identification Form

Blood processing

- Sterile Pasteur pipettes
- 5 ml pipettes
- 10 mL syringes
- 16G sterile needles (optional, sterile Pasteur pipettes can be used instead)
- 0.8 micron filters (Merck Millipore #SLAA033SS)
- 15 mL propylene tubes
- 1 mL working volume cryovials
- Sterile Phosphate Buffered Saline, without magnesium and calcium
- 1 mL micropipettes
- 1 mL micropipette tips with filter

Blood collection venipuncture procedure



1. Preparation

- Prepare the blood collection kit.
- Confirm participant identity before label the tubes.

2. Venipuncture

- Assess participant disposition.
- Collect blood using your institution's recommended standard procedure for venipuncture.

3. Blood collection

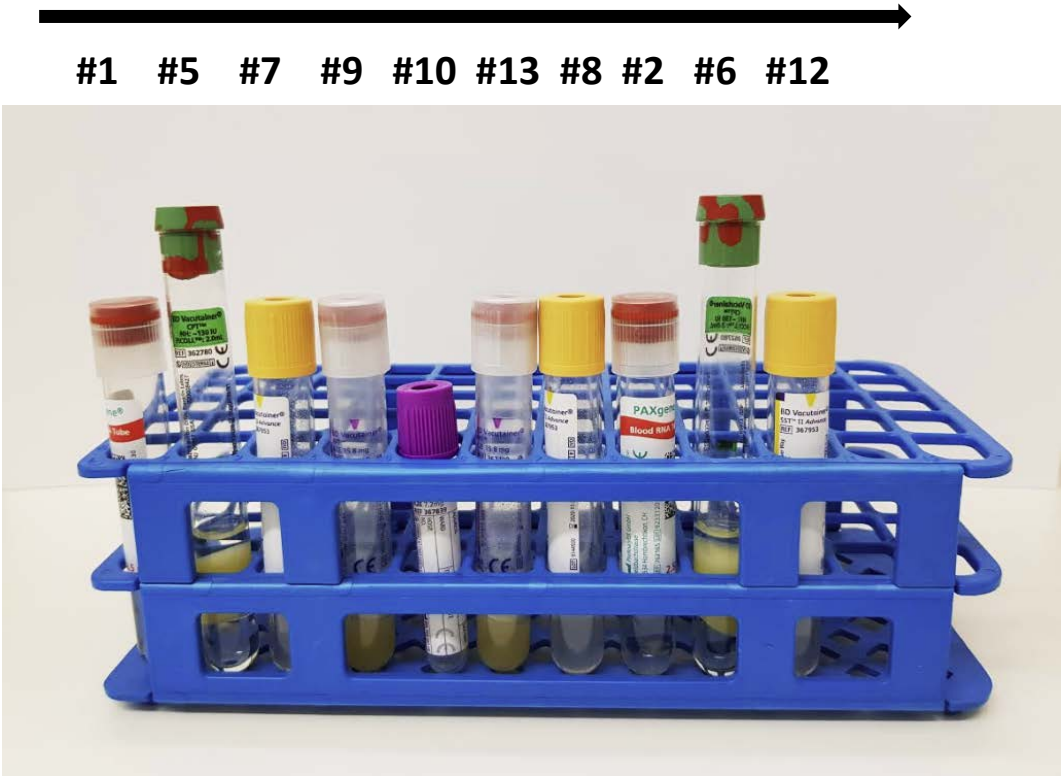
Respect the order of tubes collection!

- Place the first tube in holder and push tube forward until tube stopper has been penetrated. **Hold tubes in a vertical position!**
- Wait until tube has filled to its stated volume and blood flow ceases.
- **Make sure the tubes are totally filled.**
- Remove tube from the holder and introduce the next vacutainer into the holder.

4. After blood collection

- Immediately invert the tube after blood collection. **Respect the number of inversions of each tube!**
- Process the blood in a timeframe of 2 hours.

Blood collection tubes per patient



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Blood collection and processing: Overview

TUBES		STORAGE	BLOOD COLLECTION		PROCESSING
			Volume	Mix by inverting	(must start within 2 hours after blood collection)
#1 **#2**		18-25ºC	2.5 mL	8-10 times	• Incubate tubes upright for at least 2h and no more than 6h at RT. • Freeze tubes at -20ºC for 24h-72h. • Storage tubes at -80ºC.
#5 **#6**		4-25ºC	8.0 mL	8-10 times	*Plasma* • Centrifuge at 1,700xRCF, with brake off, for 30 min at RT. • Aspirate plasma and divide it into 500 µL cryovials aliquots. • Freeze immediately at -80ºC. *PBMC* • Collect the cell layer with a Pasteur Pipette and transfer to a 15 mL polypropylene tube. • Refill tube with 3ml PBS, pipet up and down, collect PBS/PBMCs with Pasteur Pipette and transfer to the polypropylene tube from step 1. • Wash with sterile PBS and centrifuge at 300 RCF for 15 min. Repeat the washing. • Freeze cells in 500 µL cryovials or 15 mL propylene tubes at -80ºC.
#7 **#8** **#12**		4-25ºC	8.5 mL	5 times	• Allow blood to clot for a minimum of 30 min. • Centrifuge at 1,100 RCF, with brake off, for 10 min at RT. • Aspirate serum with a needle or sterile Pasteur pipette. • Filter the serum through a 0.8 µm filter into a 15 mL propylene tube. • Divide serum into 500 µL cryovials aliquots. • Freeze immediately at -80ºC.
#9 **#13**		4-25ºC	8.5 mL	8-10 times	• Centrifuge at 1,100 RCF, with the brake off, for 10 minutes at RT. • Aspirate plasma with a needle or sterile Pasteur pipette. • Filter plasma through a 0.8 µm filter into a 15 mL propylene tube. • Divide plasma into 500 µL cryovials aliquots. • Freeze immediately at -80ºC.
#10		18-25ºC	4.0 mL	8-10 times	• Divide blood into 500 µL cryovials aliquots. • Freeze directly at -80ºC.