ESMI

European Spinocerebellar Ataxia Type 3/Machado-Joseph Disease Initiative

# Manual for Biosample collection

JPND research

EU Joint Programme – Neurodegenerative Disease Research

***Written by***

Magda M. Santana, PharmD, PhD

***Revised by***

Luís Pereira de Almeida, PharmD, PhD

Olaf Riess, MD, PhD

Jeannette Huebener-Schmid, PhD

Manuela Lima, PhD

Thomas Klockgether, MD, PhD

Jon Infante, MD

Kathrin Reetz, MD

Sandra Kuhs

Laetitia Gaspar

**December 2016**

Second version

# CONTENT


**1. STUDY INFORMATION AND DESCRIPTION ..... 7**

1.1. Purpose ..... 7

1.2. Research centers and groups ..... 9

**2. PREPARATION OF BIOSAMPLE COLLECTION ..... 13**

2.1. Participants and visits ..... 13

2.2. Sample description ..... 13

2.3. Collection tubes ..... 14

2.4. Sample labeling ..... 14

2.5 Sample identification forms ..... 15

2.6 Material and equipment ..... 16

**3. COLLECTION OF BLOOD BIOSAMPLES ..... 17**

3.1 Participants preparation ..... 17

3.2 Time for blood collection ..... 17

3.3 Venipuncture procedure ..... 17

3.4 Order of tube collection ..... 17

3.5 Number of tubes inversions ..... 18

3.6 Reporting ..... 19

**4. PROCESSING OF BLOOD BIOSAMPLES ..... 19**

4.1 PAXGene Tubes (#1, #2, #3 and #4) ..... 19

4.2 Cell Preparation Tubes (#5 and #6) ..... 19

4.3 Serum Separator Tubes (#7 and #8) ..... 20

4.4 Plasma Preparation Tube (#9) ..... 21

4.5 Plasma EDTA Tube (#10) ..... 21

**5. COLLECTION AND PROCESSING OF CSF BIOSAMPLES ..... 22**

5.1 Participants preparation ..... 22

5.2 Lumbar puncture and CSF collection ..... 22

5.3 CSF sample processing ..... 22

5.4 Reporting ..... 23

**6. COLLECTION OF FIBROBLAST SAMPLES ..... 24**

**7. STORAGE OF BIOSAMPLES ..... 25**

**8. SAMPLE COLLECTION AT EACH RESEARCH CENTER ..... 26**

**9. ANNEXES ..... 27**

# 1. STUDY INFORMATION AND DESCRIPTION

## 1.1. 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. To build up such a cohort, the European Spinocerebellar Ataxia Type3/Machado Joseph Disease Initiative (ESMI) will bring together existing preclinical and patient cohorts and will continuously enlarge them by enrolling new participants. Whereas validated clinical outcome measures for ataxia have been developed and are accepted by regulatory authorities, there is an almost complete lack of biomarkers for SCA3. Availability of validated biomarkers, however, is an essential precondition for proof of concept studies. Therefore, development and validation of innovative disease biomarkers will be a major focus of this project.

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 European Spinocerebellar Ataxia Type3/Machado Joseph Disease Initiative.

## 1.2. Research centers and groups

### 1.2.1 Contacts

*A. Partner Centers*

| **Research Center** | German Center for Neurodegenerative Diseases (DZNE)<br>Bonn, Germany |
|---|---|
| | **Coordinator (Partner 1)** |
| **Name** | Klockgether, Thomas |
| **Position** | Director of Clinical Research |
| **Address** | Sigmund-Freud-Str. 25, D-53105 Bonn, Germany |
| **Phone/Fax** | +49 228 287 15736 / +49 228 287 15024 |
| **e-mail** | klockgether@uni-bonn.de |

| **Research Center** | University College London, Institute of Neurology (UCL)<br>London, UK |
|---|---|
| | **Partner 2** |
| **Name** | Giunti, Paola |
| **Position** | Principal Clinical Research Associate/Head of the Ataxia Centre |
| **Address** | Queen Square |
| **Phone/Fax** | +44 845 155 5000 723153 / +44 207 2785616 |
| **e-mail** | p.giunti@ucl.ac.uk |

| **Research Center** | Fundação Gaspar Frutuoso, University of Azores (FGF)<br>Ponta Delgada, Azores, Portugal |
|---|---|
| | **Partner 3** |
| **Name** | Lima, Manuela |
| **Position** | Associate Professor with Aggregation, Department of Biology |
| **Address** | Campus Universitário de Ponta Delgada, 9560-801 Ponta Delgada, Azores, Portugal |
| **Phone/Fax** | +351 296 650 118 |
| **e-mail** | maria.mm.lima@uac.pt |

| **Research Center** | CNC - Center for Neuroscience and Cell Biology, University of Coimbra (CNC)<br>Coimbra, Portugal |
|---|---|
| | **Partner 4** |
| **Name** | Pereira de Almeida, Luis |
| **Position** | Principal Investigator and Vice-President (CNC);<br>Tenured Assistant Professor (Faculty of Pharmacy) |
| **Address** | Rua Larga, 3004-504 Coimbra, Portugal |
| **Phone/Fax** | +351 966 337 482 |
| **e-mail** | luispa@cnc.uc.pt |

| **Research Center** | University of Tübingen (UT)<br>Tübingen , Germany |
|---|---|
| | **Partner 5** |
| **Name** | Riess, Olaf |
| **Position** | Head, Department for Medical Genetics and Applied Genomics |
| **Address** | Calwer Straße 7, 72076 Tübingen, Germany |
| **Phone/Fax** | +49 7071 29 76458 |
| **e-mail** | Olaf.Riess@med.uni-tuebingen.de |

| **Research Center** | Radboud University (RU)<br>Nijmegen, Netherlands |
|---|---|
| | **Partner 6** |
| **Name** | van de Warrenburg, Bart |
| **Position** | Associate Professor, Department of Neurology |
| **Address** | Postbus 9100, 6500 HB NIJMEGEN, Netherlands |
| **Phone/Fax** | +31 243 613 396 |
| **e-mail** | bart.vandeWarrenburg@radboudumc.nl |

*B. Associated recruitment centers*

| **Research Center** | Universitätsklinikum der RWTH Aachen (UKA)<br>Aachen, Germany |
|---|---|
| **Name** | Kathrin Reetz |
| **Position** | Professor for Multimodal Imaging in Neurodegenerative Diseases |
| **Address** | Pauwelsstraße 30, 52074 Aachen |
| **Phone/Fax** | Tel + 49 241 80 85522 |
| **e-mail** | kreetz@ukaachen.de |

| **Research Center** | Universitätsklinikum Essen (UKE)<br>Essen, Germany |
|---|---|
| **Name** | Dagmar Timmann |
| **Position** | Professor of Clinical-Experimental Neurology |
| **Address** | Hufelandstr. 55, 45147 Essen |
| **Phone/Fax** | +49 201 723 2180 |
| **e-mail** | Dagmar.Timmann-Braun@uni-duisburg-essen.de |

| **Research Center** | Universitätsklinikum Frankfurt (UKF)<br>Frankfurt, Germany |
|---|---|
| **Name** | Jun-Suk Kang |
| **Position** | Working group leader, Movement Disorders |
| **Address** | Haus 18, EG, Theodor-Stern-Kai 7, 60590 Frankfurt |
| **Phone/Fax** | +49 69 6301 4318 |
| **e-mail** | jun-suk.kang@em.uni-frankfurt.de |

| Research Center | University Hospital Marques de Valdecilla (UHMV)<br>Cantabria, Spain |
|---|---|
| **Name** | Jon Infante |
| **Position** | Associate Professor, Section leader, Department of Neurology |
| **Address** | Avda. Cardenal Herrera Oria s/n. 39011 Santander, Cantabria |
| **Phone/Fax** | |
| **e-mail** | jinfante@humv.es |

| Research Center | University Medical Center Groningen (UMCG),<br>Groningen, Netherlands |
|---|---|
| **Name** | Jeroen J. de Vries |
| **Position** | Neurologist, Chef de Policlinique<br>Neurodegenerative & Movement Disorders |
| **Address** | Hanzeplein 1, 9713 GZ Groningen |
| **Phone/Fax** | +31 50 3613500 |
| **e-mail** | j.j.de.vries01@umcg.nl |

### 1.2.2 Responsibilities

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

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

## 2. PREPARATION OF BIOSAMPLE COLLECTION

### 2.1. Participants and visits

Samples will be collected three times: at a baseline visit (1) and in two follow-up visits, after one (2) and two years (3) of baseline visit. If samples cannot be collected at the same time of clinical evaluation, they should be collected within a month.

### 2.2. Sample description

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

**Table 1.** Sample description.

| Sample | | Collection tube | | Number x Volume | Purpose | Baseline visit | Follow up visit(s) |
|---|---|---|---|---|---|---|---|
| **Blood** | **Whole blood** | #1<br>#2<br>#3<br>#4 | PAXgene RNA | 4 x 2.7 mL | RNA<br>(RNAseq/qPCR) | X | X |
| | **PBMC** | #5<br>#6 | Cell Preparation Tube (CPT) | 2 x 8.0 mL | Protein<br>RNA | X | X |
| | **Serum** | #7<br>#8 | Serum Separator Tube (SST) | 2 x 8.5 mL | Exosomes<br>(Protein/RNA) | X | X |
| | **Plasma** | #9 | Plasma Preparation Tube (PPT) | 1 x 8.5 mL | Exosomes<br>(RNA) | X | X |
| | **Whole blood** | #10 | EDTA Tube | 1 x 4.0 mL | DNA | X | - |
| **Cerebrospinal Fluid** | | #11 | Polypropylene Tube | 1 x 15 mL | qPCR<br>Protein<br>Exosomes | X | X |
| **Fibroblasts** | | #12 | Polypropylene Tube | Small biopsy | iPSC | One time during study course | |

## 2.3. 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 present 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<br>#2<br>#3<br>#4 | PAXgene RNA | BD #762165 | | RNA stabilizing agent | 18-25°C |
| | **PBMC** | #5<br>#6 | Cell Preparation Tube (CPT) | BD #362780 | | Sodium Heparin/Ficoll | 18-25°C |
| | **Serum Plasma** | #7<br>#8 | Serum Separator Tube (SST) | BD #367953 | | Clot Activator/ Gel | 4-25°C |
| | | #9 | 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 | - |

## 2.4. Sample labeling

### 2.4.1 Collection tubes labeling

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

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

The following advertisements should be followed when labelling the tubes:

- Confirm patient identity just before collect the blood
- Place label vertical on the tube
- Avoid misalign labeling
- Ensure the label is completely adhered to the tube.

An example of correct collection tubes labeling can be found in Annex I.

### 2.4.2 Aliquots labeling

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

Each cryovial should be unequivocally labelled before blood processing.

**Each center will use their local labeling system**, but efforts must 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).

### 2.4.3 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 sample can be found in Annex II.

Every time a sample circulate between centers it must be accompanied by a Sample Sheet (Annex III) that corresponds the ESMI biosample code to the local biosample code/labeling.

## 2.5 Sample identification forms

The sample identification forms present in Annex IV (Blood Sample Identification Form), V (CSF Sample Identification Form) and VI (Fibroblasts Sample Identification Form) must be filled each time a biosampling is performed from a participant. Sample identification form should be properly archived at each center and most relevant information contained in the form will be further available at the clinical database.
The sample identification forms contain the following information:

- Research center
- Number of the visit (first, second or third)
- Date of collection
- Time of collection
- Identification number of the sample/patient
- Gender and age of the patient
- Number of repeats
- Disease onset
- SARA score

- Time of last meal
- Number of tubes collected
- Any occurrence during collection

To have a written record about each sample and aliquot, the back of the Sample Identification Form has a table that might be filled with the following information:

- ESMI biosample code (without patient ID and nº of visit, because it is written in front page)
- Center biosample code
- Volume of incomplete aliquot
- Copy of label stickers
- Errors detected during process

This latter information is not mandatory as long as each center could be able to give a correct correspondence between the ESMI biosample code and the center biosample code. This information will only be shared between centers, if necessary.

## 2.6 Material and equipment

### 2.6.1 Material

Collection tubes and any other clinical materials needed for biosample collection 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 VIII.

### 2.6.2 Equipment

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

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

## 3. COLLECTION OF BLOOD BIOSAMPLES

### 3.1 Participants preparation

Efforts must be made to ensure the procedure is easy and painless as possible. Technicians must remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The ESMI study collects approximately 56 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 5 – 6 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.

### 3.2 Time for blood collection

Blood samples should be collected during morning between 8:00 and 12:00, preferably fasting. The time of last meal must be recorded in the Sample Identification Form.

### 3.3 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 VII for a workflow of venipuncture procedure.

### 3.4 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 2.7 mL
#2. PAX Gene RNA Tube 2.7 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
#3. PAX Gene RNA Tube 2.7 mL
#4. PAX Gene RNA Tube 2.7 mL
#6. Cell Preparation Tube 8.0 mL
#8. Serum Separator Tube 8.5 mL

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

The number of tubes collected must be registered in the Blood Sample Identification Form (fill the check list with X for each tube collected).

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

### 3.5 Number of tubes inversions

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

The number of inversions of each specific tube must 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 color | Number of inversions |
|---|---|---|---|
| **#1**<br>**#2**<br>**#3**<br>**#4** | PAXgene RNA | | 8-10 times |
| **#5**<br>**#6** | Cell Preparation Tube (CPT) | | 8-10 times |
| **#7**<br>**#8** | Serum Separator Tube (SST) | | 5 times |
| **#9** | Plasma Preparation Tube (PPT) | | 8-10 times |
| **#10** | Plasma EDTA | | 8-10 times |

### 3.6 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 must be reported (ex: hemolysis during collection, timeframe for blood processing not respected, sample contamination, etc)

## 4. PROCESSING OF BLOOD BIOSAMPLES

**The blood processing must 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 must be reported in sample identification form.

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

### 4.1 PAXGene Tubes (#1, #2, #3 and #4)

- 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.

### 4.2 Cell Preparation Tubes (#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 following figure).

*For plasma collection*

- Aspirate the plasma without disturbing the cell layer plasma and divide it into 1 mL cryovials aliquots. Freeze immediately at -80ºC.
  Note: aliquots less than 1 mL might be saved or discarded.

*For PBMC collection*

- Collect cell layer with a sterile Pasteur Pipette and transfer to a 15 mL polypropylene tube.
- 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 1 mL 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.

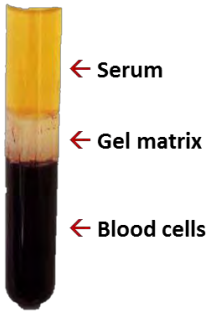
  Option 2:
  - discard the supernatant removing all the PBS from the cell pellet;
  - Freeze 15 mL propylene tube with cell pellet at -80ºC.

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

### 4.3 Serum Separator Tubes (#7 and #8)

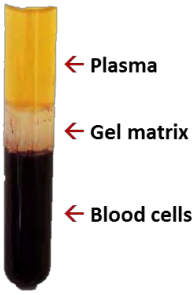
- Allow blood to clot in a vertical position for a minimum of 30 minutes and not more than 2 hours.
- Centrifuge the tube in a balanced, swing-out rotor type centrifuge at room temperature at 1,100 RCF for 10 minutes, with the brake off.
- Remove the BD Hemogard™ Closure.
- Aspirate the serum layer into a 10 mL syringe using a 16G needle (see following figure). 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 1 mL cryovials aliquots and freeze immediately at -80ºC.
  Note: aliquots less than 1ml might be saved or discarded.




### 4.4 Plasma Preparation Tube (#9)

- 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 1 mL cryovials aliquots and freeze immediately at -80ºC.
  Note: aliquots less than 1 mL might be saved or discarded.




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

### 4.5 Plasma EDTA Tube (#10)

- Divide blood into 1 mL cryovials aliquots.
  Note: aliquots less than 1 mL might be saved or discarded.
- Freeze directly at -80ºC.

A resume table for blood collection and processing can be found in Annex X.

## 5. COLLECTION AND PROCESSING OF CSF BIOSAMPLES

### 5.1 Participants preparation

Each participant must 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 must 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.

### 5.2 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).

### 5.3 CSF sample processing

**The CSF processing must 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 must be reported in sample identification form.

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 1.0 mL cryovials aliquots.
- Freeze immediately at -80ºC.

### 5.4 Reporting

The CSF sample identification should be filled immediately after CSF collection and processing. Any deviation from this manual or any occurrence during collection and processing must be reported.

## 6. COLLECTION OF FIBROBLAST SAMPLES

Skin biopsies should be performed according to each center recommended procedure.

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

The protocol used should be described in the back of the sample preparation form. This information will only be shared between centers, if necessary.

## 7. STORAGE OF BIOSAMPLES

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

The clinical database will provide information about the available samples, their storage sites and the clinical data associated with the samples.

**Instructions for biosample exchange between centers and further analysis will be found in the "Manual of biosample analysis" (under preparation).**

## 8. SAMPLE COLLECTION AT EACH RESEARCH CENTER

Specific alterations to the protocol will be performed by each research center, in order to adapt biosampling collection to local conditions, without compromise 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 |
|---|---|---|---|---|
| ***Partner centers*** | | | | |
| **DZNE, Bonn, Germany** | • Jennifer Faber Jennifer.Faber@dzne.de | All tubes | According to manual | Local |
| **UCL, London, UK** | • Paola Giunti p.giunti@ucl.ac.uk<br>• Hector Garcia-Moreno h.garcia-moreno@ucl.ac.uk | All tubes | According to manual | Local (with limitations) |
| **FGF, Azores, Portugal** | • Mafalda Raposo mafalda.sb.raposo@uac.pt<br>• Manuela Lima maria.mm.lima@uac.pt | All tubes | According to manual | Local |
| **CNC, Coimbra, Portugal** | • Magda Santana mmsantana@uc.pt | All tubes | According to manual | Local |
| **UT, Tübingen, Germany** | • Jeannette Huebener Jeannette.Huebener@med.uni-tuebingen.de | All tubes | According to manual | Local |
| **RU, Nijmegen, Netherland** | • Judith VanGaalen Judith.vanGaalen@radboudumc.nl | PaxGene tubes, SST, PST, EDTA | According to manual, except for SST and PST (without filtration step) | Local |
| ***Associated centers*** | | | | |
| **UKA, Aachen, Germany** | • Kathrin Reetz kreetz@ukaachen.de | All tubes | According to manual | DZNE, Bonn, Germany |
| **UKE, Essen, Germany** | • Dagmar Timmann Dagmar.Timmann-Braun@uni-duisburg-essen.de | Only PaxGene tubes | Tubes will be send to Bonn in 24h at 4ºC; once arrived, processing will be performed according to manual | DZNE, Bonn, Germany |
| **UKF, Frankfurt, Germany** | • Jun-Suk Kang Jun.suk.kang@em.uni-frankfurt.de | Only PaxGene tubes | Tubes will be send to Bonn in 24h at 4ºC; once arrived, processing will be performed according to manual | DZNE, Bonn, Germany |
| **UHMV, Cantabria, Spain** | • Jon Infante jinfante@humv.es | All tubes | According to manual | Local |
| **UMCG, Groningen, Netherland** | • Jeroen de Vries j.j.de.vries01@umcg.nl | All tubes | According to manual | Local |

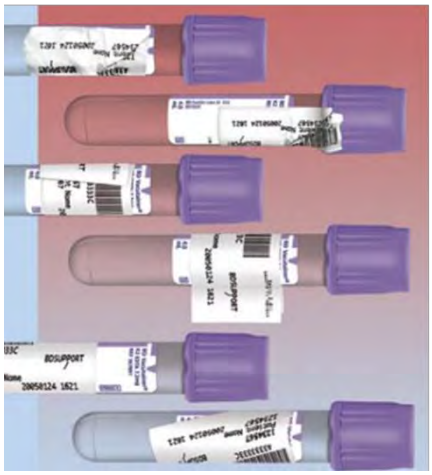
For any question please contact:

**Magda Santana**

mmsantana@uc.pt

# 9. ANNEXES

**ANNEX I – Collection tubes labelling**

| INCORRECT | CORRECT |
| --- | --- |
|  |  |

**ANNEX II – General ESMI biosample codes**

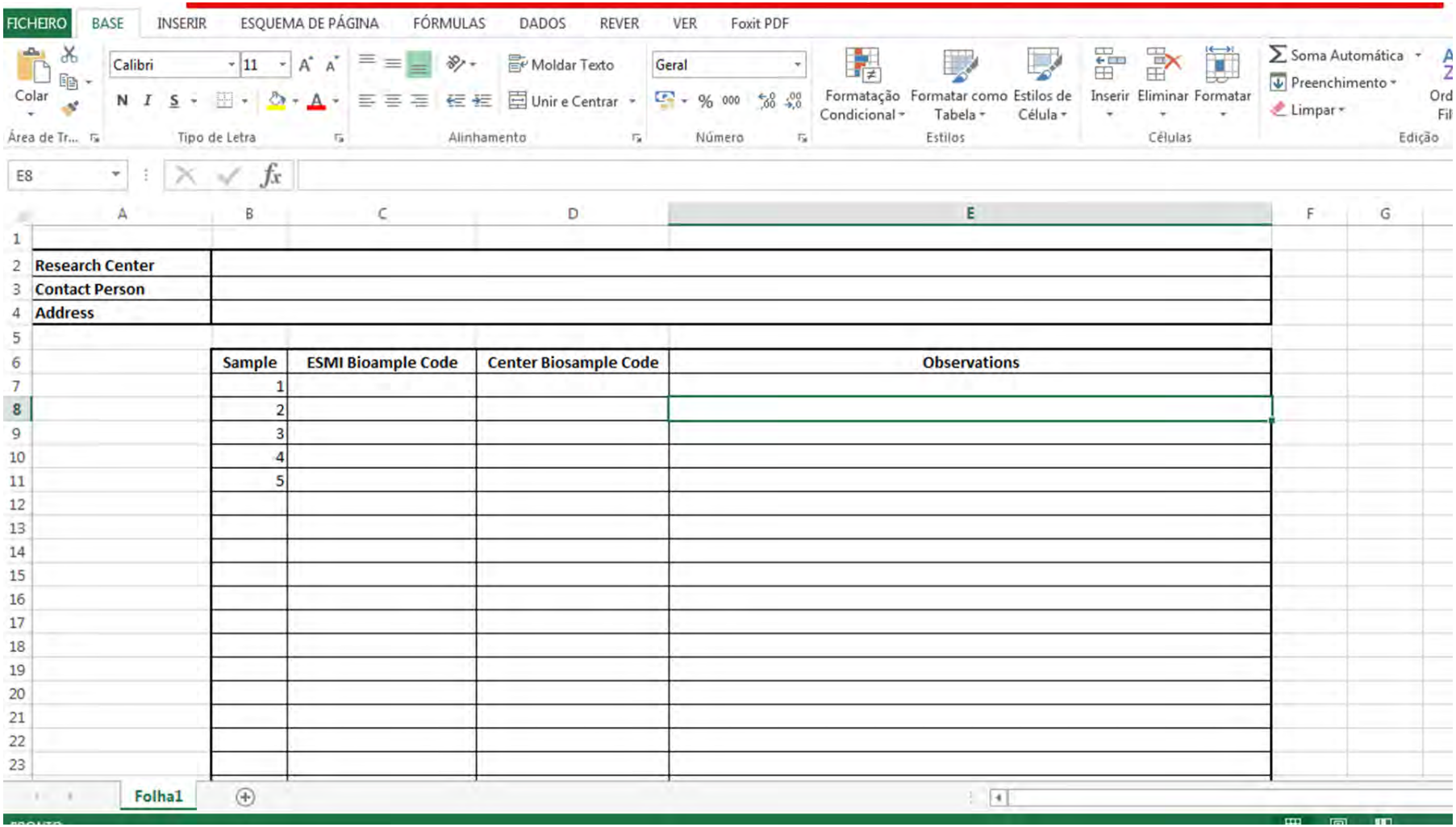
***Code generation:***

| **ESMI Patient ID / Nº of visit: Nº of original blood tube: Nº of the aliquot** |
|---|

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

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

| Nº of 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 | 3475490/ 15 | 3475490/ 150 |
| | | | Plasma aliquot 1 | 3475490/ 15 | 3475490/ 151 |
| | | | Plasma aliquot 2 | 3475490/ 15 | 3475490/ 152 |
| | | | Plasma aliquot 3 | 3475490/ 15 | 3475490/ 153 |
| | | #6 | PBMCs | 3475490/ 16 | 3475490/ 160 |
| | | | Plasma aliquot 1 | 3475490/ 16 | 3475490/ 161 |
| | | | Plasma aliquot 2 | 3475490/ 16 | 3475490/ 162 |
| | | | Plasma aliquot 3 | 3475490/ 16 | 3475490/ 163 |
| | SST | #7 | Serum aliquot 1 | 3475490/ 17 | 3475490/ 171 |
| | | | Serum aliquot 2 | 3475490/ 17 | 3475490/ 172 |
| | | | Serum aliquot 3 | 3475490/ 17 | 3475490/ 173 |
| | | | Serum aliquot 4 | 3475490/ 17 | 3475490/ 174 |
| | | #8 | Serum aliquot 1 | 3475490/ 18 | 3475490/ 181 |
| | | | Serum aliquot 2 | 3475490/ 18 | 3475490/ 182 |
| | | | Serum aliquot 3 | 3475490/ 18 | 3475490/ 183 |
| | | | Serum aliquot 4 | 3475490/ 18 | 3475490/ 184 |
| | PPT | #9 | Plasma aliquot 1 | 3475490/ 19 | 3475490/ 191 |
| | | | Plasma aliquot 2 | 3475490/ 19 | 3475490/ 192 |
| | | | Plasma aliquot 3 | 3475490/ 19 | 3475490/ 193 |
| | | | Plasma aliquot 4 | 3475490/ 19 | 3475490/ 194 |
| | EDTA | #10 | Aliquot 1 | 3475490/ 110 | 3475490/ 1101 |
| | | | Aliquot 2 | 3475490/ 110 | 3475490/ 1102 |
| | | | Aliquot 3 | 3475490/ 110 | 3475490/ 1103 |
| | | | Aliquot 4 | 3475490/ 110 | 3475490/ 1104 |
| | CSF | #11 | Aliquot 1 | 3475490/ 111 | 3475490/ 1111 |
| | | | Aliquot 2 | 3475490/ 111 | 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 | 3475490/ 25 | 3475490/ 250 |
| | | | Plasma aliquot 1 | 3475490/ 25 | 3475490/ 251 |
| | | | Plasma aliquot 2 | 3475490/ 25 | 3475490/ 252 |
| | | | Plasma aliquot 3 | 3475490/ 15 | 3475490/ 153 |
| | | #6 | PBMCs | 3475490/ 26 | 3475490/ 260 |
| | | | Plasma aliquot 1 | 3475490/ 26 | 3475490/ 261 |
| | | | Plasma aliquot 2 | 3475490/ 26 | 3475490/ 262 |
| | | | Plasma aliquot 3 | 3475490/ 26 | 3475490/ 263 |
| | SST | #7 | Serum aliquot 1 | 3475490/ 27 | 3475490/ 271 |
| | | | Serum aliquot 2 | 3475490/ 27 | 3475490/ 272 |
| | | | Serum aliquot 3 | 3475490/ 27 | 3475490/ 273 |
| | | | Serum aliquot 4 | 3475490/ 27 | 3475490/ 274 |
| | | #8 | Serum aliquot 1 | 3475490/ 28 | 3475490/ 281 |
| | | | Serum aliquot 2 | 3475490/ 28 | 3475490/ 282 |
| | | | Serum aliquot 3 | 3475490/ 28 | 3475490/ 283 |
| | | | Serum aliquot 4 | 3475490/ 28 | 3475490/ 284 |
| | PPT | #9 | Plasma aliquot 1 | 3475490/ 29 | 3475490/ 291 |
| | | | Plasma aliquot 2 | 3475490/ 29 | 3475490/ 292 |
| | | | Plasma aliquot 3 | 3475490/ 29 | 3475490/ 293 |
| | | | Plasma aliquot 4 | 3475490/ 29 | 3475490/ 294 |
| | EDTA | #10 | N/A | N/A | N/A |



| | |
|---|---|
| **Research Center** | |
| **Contact Person** | |
| **Address** | |

| Sample | ESMI Bioample Code | Center Biosample Code | Observations |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |

**ANNEX IV – Blood identification form**

## BLOOD SAMPLE IDENTIFICATION FORM

**Research center**

Label with patient ID (optional)

**Patient Identification Number** _____

**Date of collection** _____ /_____ /_____

**Time of collection** _____

**Number of the visit** Baseline (1) _____ Second (2) _____ Third (3)_____

**Gender** Male____ Female____

**Age** _____

**Disease onset** _____

**Number of repeats** _____

**SARA Score** _____

**Time of last meal** _____

**Blood Collection**

| Collection tubes | Check list | Number of aliquots |
|---|---|---|
| 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: |
| 6. Cell Preparation Tube | | PBMCs: Plasma: |
| 7. Serum Separator Tube | | Serum: |
| 8. Serum Separator Tube | | Serum: |
| 9. Plasma Preparation Tube | | Plasma: |
| 10. Plasma EDTA Tube | | Blood: |

**Others:**

| ESMI Code | Center Code | Observations<br>(ex: volume of incomplete aliquots, copy of label stickers, errors detected that need to be recorded) |
|---|---|---|

**ANNEX V – CSF identification form**

# CSF SAMPLE IDENTIFICATION FORM

| Research center | Label with patient ID (optional) |
|---|---|

**Patient Identification Number** _____

**Date of collection** _____ / ____ /____

**Time of collection** _____

**Number of the visit** Baseline (1) ___ Second (2)___ Third (3)___

**Gender** Male____ Female____

**Age** _____

**Disease onset** _____

**Number of repeats** _____

**SARA Score** _____

**Volume collected** _____

**Time of last meal** _____

**Others:**

| ESMI Code | Center Code | Observations |
|---|---|---|
| | | |

**ANNEX VI – Fibroblasts identification form**

# FIBROBLASTS SAMPLE IDENTIFICATION FORM

**Research center**

Label with patient ID (optional)

**Patient Identification Number** _____

**Date of collection** _____ / ____ /____

**Time of collection** _____

**Number of the visit** Baseline _____ Second _____ Third_____

**Gender** Male____ Female____

**Age** _____

**Disease onset** _____

**Number of repeats** _____

**SARA Score** _____

**Punch site** _____

**Punch diameter** _____ **mm**

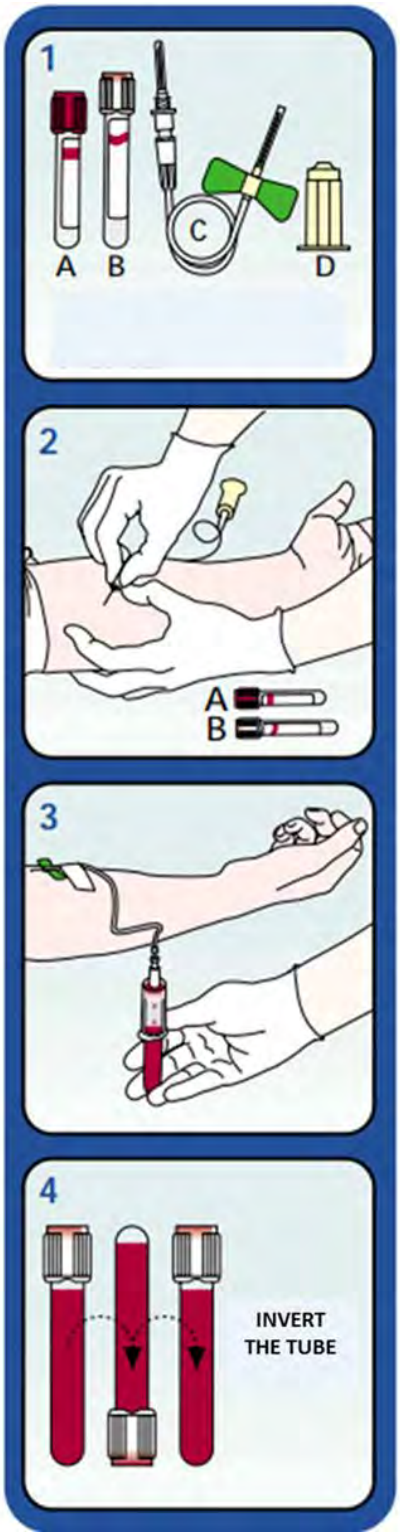
**Collection Medium** _____

**Others:**

| ESMI Code | Center Code | Observations |
|---|---|---|
| | | |

**Protocol:**

**ANNEX VII – 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.

**ANNEX VIII – Material and reagents**

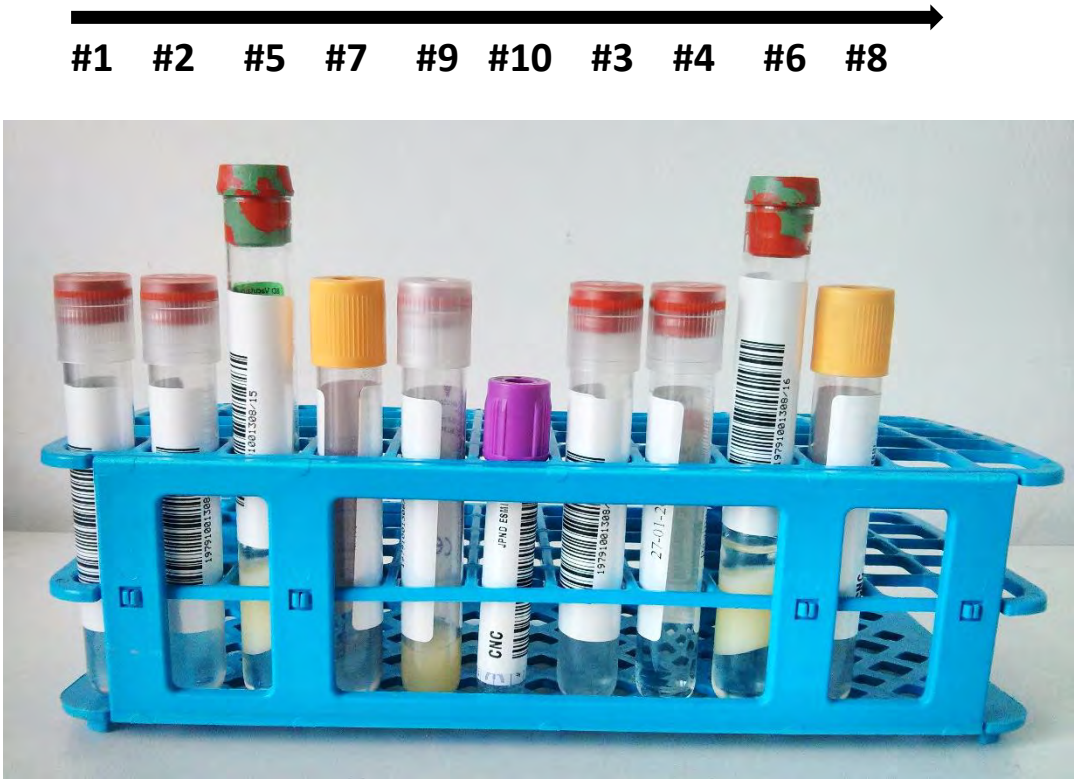
**Blood collection**

- 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)
    - 2 x Serum Separator Tube 8.5 mL (BD #367953)
    - 1 x Plasma Preparation Tube 8.5 mL (BD #362799)
    - 1 x Plasma Preparation Tube 4.0 mL (BD #367839)
    - 4 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
- 16 G 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

**ANNEX IX – Blood collection tubes per patient**



| TUBES | | STORAGE | BLOOD COLLECTION | | PROCESSING |
|---|---|---|---|---|---|
| | | | Volume | Mix by inverting | (within 2 hours after blood collection) |
| **#1**<br>**#2**<br>**#3**<br>**#4** |  | 4ºC | 3.0 mL | 10 times | • Incubate tubes upright for at least 2h and no more than 6h at RT.<br>• Freeze tubes at -20ºC for 24h-72h.<br>• Storage tubes at -80ºC. |
| **#5**<br>**#6** |  | 4-25ºC | 8.0 mL | 8-10 times | *Plasma*<br>• Centrifuge at 1,700xRCF, with brake off, for 30 min at RT.<br>• Aspirate plasma and divide it into 1mL cryovials aliquots.<br>• Freeze immediately at -80ºC.<br>*PBMC*<br>• Collect the cell layer with a Pasteur Pipette and transfer to a 15mL polypropylene tube.<br>• Wash with sterile PBS and centrifuge at 300 RCF for 15 min. Repeat the washing.<br>• Freeze cells in 1mL cryovials or 15ml propylene tubes at -80ºC. |
| **#7**<br>**#8** |  | 4-25ºC | 8.5 mL | 5 times | • Allow blood to clot for a minimum of 30 min.<br>• Centrifuge at 1,100 RCF, with brake off, for 10 minutes at RT.<br>• Aspirate serum with a needle or sterile Pasteur pipette.<br>• Filter the serum through a 0.8 µm filter into a 15ml propylene tube.<br>• Divide serum into 1mL cryovials aliquots.<br>• Freeze immediately at -80ºC. |
| **#9** |  | 4-25ºC | 8.5 mL | 8-10 times | • Centrifuge at 1,100 RCF, with the brake off, for 10 minutes at RT.<br>• Aspirate plasma with a needle or sterile Pasteur pipette.<br>• Filter plasma through a 0.8 µm filter into a 15ml propylene tube.<br>• Divide plasma into 1mL cryovials aliquots.<br>• Freeze immediately at -80ºC. |
| **#10** |  | 18-25ºC | 4.0 mL | 8-10 times | • Divide blood into 1mL cryovials aliquots.<br>• Freeze directly at -80ºC. |